ACTA PHYSIOLOGICA SCANDINAVICA
VOL. 47 . SUPPLEMENTUM 165

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BY

OLE K. ALBRECHTSEN

AARHUS
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Translated from Danish
by
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Aarhus

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FROM THE BIOLOGICAL INSTITUTE
OF THE CARLSBERG FOUNDATION, COPENHAGEN,
DENMARK

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Denne afhandling er i forbindelse med omstående tidligere publicerede afhandlinger af det lægevidenskabelige fakultet ved Københavns universitet antaget til offentlig at forsvares for den medicinske doktorgrad.

København, den 30. april 1959.

FR. THERKELSEN
h. a. dec.

PRINTED IN DENMARK BY AARHUUS STIFTSBOGTRYKKERIE

PREVIOUSLY PUBLISHED PAPERS

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TO MY PARENTS

PREFACE

The experimental studies forming the basis of this monograph were performed during my appointment as a research associate at the Biological Institute of the Carlsberg Foundation, Copenhagen, during the years 1954–1956. I am greatly indebted to the head of the Institute at that time, the late *Dr. Albert Fischer,* for the excellent working facilities provided.

For a number of years, several members of the staff of the Institute have been engaged in the studies on the coagulation of blood and the fibrinolytic enzyme system in the organism under the leadership of *Dr. Tage Astrup.* The present work forms part of this research project. I wish to express my deep gratitude to *Dr. Astrup,* under whose direction my studies were begun, for his valuable suggestions in numerous discussions and for his constructive criticism and unfailing interest during the execution of the work.

Thanks are also due to my colleagues and other members of the staff of the Institute for excellent collaboration and for advice and guidance.

Some of the experiments were performed in collaboration with *Drs. Jørgen Rasmussen, Ole Storm, Jørn Hess Thaysen and Dyre Trolle,* to whom I wish to express my indebtedness for the help provided in the solution of the problems concerned.

I also want to thank *Miss Mona Rimestad* for her excellent technical assistance.

Financial support for the investigations was provided by the *Josiah Macy Jr. Foundation,* New York, and the *National Danish Association against Rheumatic Diseases* through grants to *Dr. Tage Astrup.* I received further support from the *Apotheker August Kongsteds Legat,* Copenhagen, for which I am grateful.

I am indebted to *Løvens kemiske Fabrik,* Copenhagen, for supplies of albino rats and sex hormones.

Finally, I owe a great debt of gratitude to my wife, *Kirsten,* for her invaluable support during the preparation of this monograph and the work which preceded it.

This survey, together with previously published papers on the subject, was submitted to the Faculty of Medicine of the University of Copenhagen in May 1958 and accepted for public defence for the degree of Doctor of Medicine in April 1959.

August, 1959.

Ole Albrechtsen.

CONTENTS

INTRODUCTION

Fibrinolysis is the process by which the organism dissolves fibrin. The problems related to fibrinolysis have been extensively studied, particularly during the last 10 years, and the results of these studies have been reported in a large series of publications.

Macfarlane and *Biggs* (1948) published the first survey of the mechanism of fibrinolysis and its importance in the organism, and this survey has later been supplemented by others (*Halse* 1948, *Permin* 1949, *Astrup* 1955 b, 1956 d, *Müllertz* 1956).

Our present concept of the fibrinolytic components and their interrelationship appear from figure 1 (after *Astrup* 1956 d), which, although still incomplete, gives an explanation of the observations made to date.

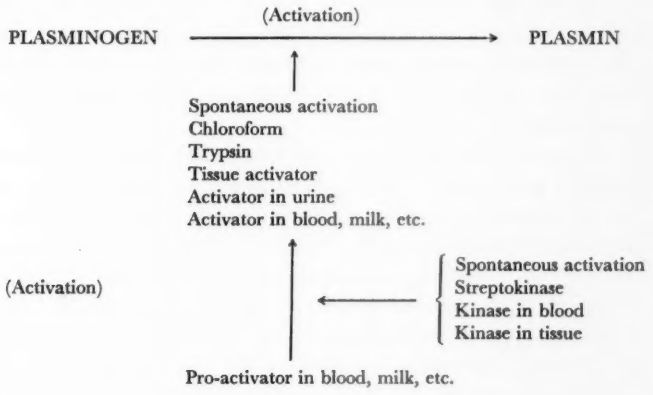

Fig. 1.—The fibrinolytic enzyme system (after *Astrup* 1956 d).

The fibrin-splitting enzyme (plasmin, fibrinolysin) is normally present in the blood as a precursor (plasminogen, profibrinolysin) (*Kaplan* 1944, *Christensen* 1945, *Christensen* and *MacLeod* 1945). The transformation of the enzymatic precursor into the active enzyme can be effected in various ways. It has thus been shown that certain tissues contain a plasminogen activator (*Astrup* and *Permin* 1947). This tissue activator is relatively stable and is not destroyed by heating to 70 or 100° C. at acid reaction (*Astrup* and *Sterndorff* 1956 a). Urine also contains a plasminogen activator (*Williams* 1951), the nature of which is not definitely known. Furthermore, plasminogen may be converted into plasmin under the influence of trypsin, by shaking with chloroform, and by a spontaneous process of an unknown nature.

In addition to plasminogen, normal blood contains a precursor of a plasminogen activator (*Müllertz* and *Lassen* 1953, *Müllertz* 1955 c). This precursor can be converted into a plasminogen activator under the influence of so-called lysokinases. It has been shown that streptokinase, a metabolic product from certain strains of haemolytic streptococci, acts as a lysokinase, and components with a similar effect have occasionally been found in the blood (*Müllertz* 1955 a, b) and in certain tissues, especially in the human kidney (*Astrup* and *Sterndorff* 1956 b). As contrasted with the plasminogen activator of the tissues, that of the blood is labile, especially at acid and alkaline reactions (*Müllertz* 1955 a). This lability renders it possible to separate these two components. A number of other body fluids, such as milk and tears, contain a plasminogen pro-activator of the same nature as that present in blood (for references, see *Astrup* 1956 c).

In addition to fibrinolytic activators, the organism contains inhibitory components against these activators. Thus, blood contains an antiplasmin and, presumably, also inhibitors against the plasminogen activators and the lysokinases. *Jacobsson* (1955) recently reviewed the inhibitors of the blood, but our knowledge of these and of the inhibitors present in certain types of tissue is still limited.

In the light of our present knowledge there are thus several possibilities of increased fibrinolytic activity in the living organism. (1) Liberation of the stable tissue activator may result in an activation of the plasminogen in the blood and in fibrin formations in the

organism. (2) Liberation of substances with lysokinase effect may result in a conversion of the pro-activator of the blood or other body fluids into a plasminogen activator of the labile type. (3) A reduction in the blood content of inhibitors may result in a shift in the regulatory mechanism in the direction of increased fibrinolysis.

The purpose of the monograph presented here is to give an account of the distribution in the organism of some of the above-mentioned components on the basis of observations previously published by others and myself. However, the fibrinolytic components in the blood are left out here, because they have recently been reviewed by *Müllertz* (1956). The fibrinolytic activity of (1) various tissues, (2) the uterus, endometrium, decidua and menstrual blood, (3) the prostate and seminal fluid, and of (4) secretions and transudates is considered in separate chapters, and a brief discussion of the biological significance of these observations is presented.

Chapter I

FIBRINOLYTIC ACTIVITY OF TISSUES

Introduction

It is an old observation that during cultivation in vitro of certain tissues from a number of different animal species, a decomposition of the fibrin occurs in the nutrient medium. This has given rise to considerable difficulties, because the cells by this process lose the support which is necessary during the growth. In order to provide optimal growth conditions for the cells it is therefore of practical importance to analyse this phenomenon in detail. A large number of studies on this problem are available. It has been shown that fibrin substrates from different animal species are digested more or less readily, and that the ability of the tissues to produce this digestion varies from species to species and from one type of tissue to another. Moreover, the tissue cultures seem to a lesser degree to digest heterologous than homologous fibrin. There has been some diversity of opinion as to whether the fibrinolytic activity of the tissues is linked up with the living cell or is a phenomenon which is characteristic of necrotic cells, and as to whether the activity varies with the growth rate. This has particularly been discussed in relation to the proteolytic properties of cancer tissue. These problems have been considered in several reviews of the literature (*Kapel* 1929, *Santesson* 1935, *Permin* 1949). However, the factors entering into the aforementioned fibrinolytic process must be determined before it is possible to analyse these phenomena in detail.

In the light of our present knowledge, the ability of the tissues to break down impure fibrin substrates may be explained by the following two processes:—

1. A direct decomposition of the fibrin by one or more proteolytic enzymes in the tissue.

2. A decomposition of the fibrin caused by the formation of proteolytic enzymes in the fibrin substrate from their precursors under the influence of activators present in the tissues.

In order to be able to distinguish between these two possibilities we must know the composition of the fibrin substrates. Several earlier erroneous conclusions are referable to lack of knowledge within this field. The knowledge of the presence of plasminogen in the fibrin substrates *(Kaplan, Christensen)* and the possibility which has now been created of destroying this by heating without a simultaneous destruction of the fibrin (*Lassen* 1952) render it possible to differentiate between the above-mentioned two processes.

1. Several investigators have demonstrated the presence of proteolytic enzymes (cathepsins) in the tissues (for references, see *Permin* (1949) and *Smith* (1951)). According to the reports, several types of cathepsins exist. Cathepsin is a term used to describe intracellular enzymes with a direct action on proteins (*Tallan, Jones* and *Fruton* 1952). These tissue enzymes have their reaction optimum at a weakly acid pH. They have been found in the liver, spleen and kidney, while the lung, heart, muscles, uterus and brain do not contain cathepsins.

The observation that a variety of animal tissues are capable of digesting fibrin from various animal species is the background for the theory that the tissues contain a fibrinolytic enzyme (fibrinolysin) (*Fleisher* and *Loeb* 1915). Several investigators, who were all able to demonstrate that tissue or tissue extracts can digest both human and animal fibrin, have later accepted this theory (*Rosenmann* 1920, 1923, 1936, *Burrows* 1927, *Kapel* 1929, *Caffier* 1930, *Santesson* 1935, *Huggins, Vail* and *Davis* 1943, *Macfarlane* and *Biggs* 1948, *Page, Glendening* and *Parkinson* 1951, and others). However, our present knowledge of the occurrence of plasminogen in these fibrin substrates necessitates that this question is subjected to further investigation.

2. *Demuth* and *v. Riesen* (1928 a, b) were the first to suggest that an activator present in the cells might be liberated during the development of cell necrosis and exert an action on an enzymatic precursor in the nutrient medium (a mixture of plasma and embryonic extract), resulting in a transformation of the precursor into an

active fibrinolytic ferment. However, they failed to prove this assumption. It was later pointed out by *Astrup* and *Darling* (1943) that the possibility exists that an activator present in the tissues is able to convert a precursor in the blood into an active protease. In tissue cultures of the Rous chicken sarcoma grown on chicken plasma, *Fischer* (1946) observed lysis of the growth medium, while a similar lysis did not occur when the plasma had been heated to 56° C. for 4 hours; nor did lysis occur when the cultures were grown on rabbit plasma. He concluded that a substance present in the tumour cells, and perhaps to a varying degree in all cell forms, on contact might be able to convert a proteolytic precursor in the homologous plasma into an active proteolytic enzyme. The proof of this conversion was furnished by *Astrup* and *Permin* (1947), who showed that a potent fibrinolytic enzyme was formed when a fibrinogen solution containing various washed tissue fragments was incubated at 37° C. for 24 hours. This enzyme did not develop when the fibrinogen solution was replaced by saline (*Permin* 1949). The plasminogen activator present in the tissues was named "fibrinokinase," a term which was later discarded and replaced by the more neutral "tissue activator of plasminogen." In addition, *Permin* (1949) showed that this activator differs from cathepsins in that it is not stimulated or inhibited by a variety of papainase activators and inhibitors (glutathione, ascorbic acid, cysteine, hydrocyanic acid and potassium bromate). In the study of fibrinolysis in tissue cultures, *Goldhaber, Cornman* and *Ormsbee* (1947) arrived at a similar conclusion. These results were later confirmed by *Permin* (1947), *Astrup* and *Permin* (1948), *Tagnon* and *Petermann* (1949 a, b), *Tagnon* and *Palade* (1950), *Lewis* and *Ferguson* (1950), *Permin* (1950), *Fantl* and *Fitzpatrick* (1950), *Loomis* (1950), *Astrup* (1951 a, b), *Astrup* and *Sterndorff* (1952 a), *Astrup* and *Stage* (1952), and others.

While certain tissues are thus able to digest untreated fibrin, this ability is lost on heating of the fibrin (*Fleisher* and *Loeb* 1915, *Fischer* 1946, *Fantl* and *Fitzpatrick* 1950). It has later been shown that the plasminogen in bovine fibrin is destroyed by heating (*Lassen* 1952), which explains the aforementioned observations.

Normal blood contains a precursor of a plasminogen activator (*Müllertz* and *Lassen* 1952). In the attempts to find an activator

of this pro-activator in the organism certain tissues have been studied (*Astrup* and *Sterndorff* 1956 b). The results of their investigation suggest that certain tissues, especially the human kidney, may contain such an activator (a lysokinase).

Fibrinolytic Inhibitors in Tissues

Certain tissues inhibit the fibrinolytic process. The nature of the inhibitory components is largely unknown. Nor is it known whether these inhibitors exert their action on plasmin, plasminogen activators or other components of the system. This cannot be investigated until it becomes possible to produce sufficiently pure preparations of the active components for experimental use.

The first investigations on fibrinolytic inhibitors in tissues (*Fleisher* and *Loeb* 1915) showed that saline emulsions from hepatic tissue inhibited the fibrin-splitting properties of other organs. Subsequent studies showed that saline extracts from a number of tissues (human lung, bovine thyroid gland, kidney, liver and heart) exerted an inhibitory action on fibrinolysis by counteracting the spontaneous fibrinolytic activity in precipitated and washed horse fibrin (*Rosenmann* 1922). It was possible to separate the active fibrinolytic components in certain tissues (spleen, lung, liver, kidney, thyroid gland and heart of the pig and ox) from the inhibitors present in these tissues by dialysis of fluid expressed from them and by washing of the precipitated globulin, because the active components (thrombolysins) in this globulin can be dissolved in saline containing sodium carbonate, whereas the inhibitors (thromboligins) are insoluble. Studies of human organs performed by *Macfarlane* and *Biggs* (1948) revealed the presence of inhibitors in saline extracts from a number of tissues (liver, kidney, spleen, adrenal, thyroid, muscles, lung, heart and brain). These components could be separated from the active fibrinolytic substances in the extracts by isoelectric precipitation, since the inhibitors were found in the albumin fraction and the active components in the globulin fraction. However, as the experimental technique used was not described in detail, the results are difficult to assess. *Tagnon* and *Petermann* (1949 a, b) and *Tagnon* and *Palade* (1950) demonstrated soluble inhibitors in the rat lung. They separated the inhibitors from the

insoluble active components by centrifugation after having ground the tissue and suspended the tissue pulp in a 30 % sugar solution. *Lewis'* and *Ferguson* (1950) arrived at similar results in studies of a number of tissues from dogs and of pulmonary tissue from the mouse, rat, guinea pig, cat, dog, ox, rabbit and man. *Page, Glendening* and *Parkinson* (1951) found fibrinolytic inhibitors in extracts from the human endometrium and placenta, but were unable to separate them from the active components present in these tissues. *Bierstedt* (1955 c) found fibrinolytic inhibitors of an unknown nature in aqueous extracts from the human liver. The character of the tissue inhibitors observed is unknown.

The first detailed studies on tissue inhibitors appeared after the observation of a specific plasmin and trypsin inhibitor in the ox lung (*Astrup* 1950). This inhibitor, which was termed pulmin, is absent in pulmonary tissue from other species (pig, rabbit, rat, mouse and man). Its inhibitory action on plasmin depends on the amounts of both plasmin and ox-lung tissue (*Astrup* 1952 a). The properties of pulmin have later been analysed by *Astrup* and *Stage* (1956), who showed that potassium thiocyanate is a specific solvent for the inhibitor, which cannot be extracted by secondary phosphate, ammonium hydroxide, sodium salicylate, dextrose, thiosulphate or pyridine, but to some extent by sodium hydroxide. The inhibitor is stable at acid reaction (pH 1–2), even at high temperatures (56 and 100° C.), but is destroyed at alkaline reaction at these temperatures, and is in this respect reminiscent of the trypsin inhibitors which have later been isolated from the pancreas (*Laskowski* and *Laskowski* 1954). According to the reports, it is low-molecular. Pulmin cannot be removed from the potassium-thiocyanate extract by precipitation with acids, whereas the tissue activator of plasminogen can be precipitated by this treatment (*Astrup* and *Albrechtsen* 1957). Hence a separation of the two components is possible. Later investigations (*Albrechtsen* 1957 b) have confirmed the absence of pulmin in pulmonary tissue from animal species other than the ox, whereas *Scevola, Novati* and *Felisati* (1954) showed that phosphate-buffer extracts from a variety of animal tissues, including that of the lung, contain a trypsin inhibitor. This applies to pulmonary tissue from the ox, guinea pig, man, rat and rabbit, to heart tissue from the guinea pig, rat, ox, rabbit and man, and to placental tissue from

the human female, guinea pig, ox, rat and rabbit. At the present time it is not possible to explain these discrepancies, but it is likely that two different inhibitors are at work. However, the method used by *Scevola, Novati* and *Felisati,* viz. homogenisation of the tissue, extraction with phosphate buffer at pH 7.4, centrifugation at 3500 r. p. m. and estimation of the activity of the extract against trypsin, does not exclude the possibility that the inhibitory effect is referable to components of the blood present in the tissue, and not to tissue inhibitors. Inhibitors of the pulmin type have been found not only in the bovine lung but also in the bovine uterus (*Albrechtsen* 1957 b), while other tissues from man and animals do not contain pulmin (*Albrechtsen* 1957 a, b). Pulmonary tissue from calf embryos does not contain pulmin (*Astrup* 1952 a) until late in foetal life (*Albrechtsen* 1957 b).

Thus, the occurrence of pulmin in various tissues is now known, but the question as to the presence of other tissue inhibitors is still unanswered. However, the investigations cited above suggest that such inhibitors exist, but that they differ from pulmin.

Methods for the Determination of the Tissue Activator of Plasminogen

An adequate method for the quantitative and qualitative determination of the content of tissue activator of plasminogen must fulfil the following requirements:—

1. The method must be specific for the plasminogen activator, and other active fibrinolytic components in the tissues must not exert any influence on the result obtained.

2. It should be possible to exclude any influence of tissue inhibitors.

3. It must be possible to exclude the presence of other components with a stimulatory or inhibitory influence on the activity estimated by the method (for example, active and inhibitory components in the blood which is present in the tissue).

4. It must be possible for the entire amount of tissue activator present in the tissues to act on the substrate used for the estimation.

5. It must be possible to exclude that factors in the substrates exert any influence on the results obtained.

All earlier methods were inadequate because one, or often more, of the aforementioned requirements were not fulfilled.

The first methods for the determination of fibrinolytic activity of tissues utilised a direct contact between the untreated tissue and the substrate used for the assay. Thus, *Halban* and *Frankl* (1910) placed tissue fragments direct on a serum substrate in a Petri dish and expressed the fibrinolytic activity by the area of the digested zone of the substrate. The method seems to have been relatively inaccurate and poorly standardised, and the results were to some extent based on individual judgment. A similar method was later used by *Caffier* (1930). *Astrup* and *Permin* (1947) and *Permin* (1947, 1949) elaborated a similar technique based on experience gained in tissue cultures. The activity was determined by measuring the area of the lysed zone which was produced by tissue fragments placed on a thin layer of bovine fibrin on the bottom of a Petri dish after incubation at 37° C. for 20 hours (the fibrin-plate method). The same method was later used by *Loomis* (1950). *Fleisher* and *Loeb* (1915) measured the time required for the lysis of a certain amount of clotted blood plasma (from various animals) produced by tissue fragments in direct contact with the plasma; the tissue fragments were either placed on the clot, or the plasma was allowed to clot around the tissue. The activity measured in all these experiments may have been caused both by plasminogen activators and by proteolytic enzymes in the tissues. Moreover, tissue inhibitors, if any, and active and inhibitory components originating from blood or other body fluids present in the tissue may also have influenced the result. In addition, there is no guarantee that the entire amount of tissue activator present in the tissue is acting on the substrate. Accordingly, these methods cannot be used for qualitative or quantitative determinations, but they have been of value in exploratory experiments.

Other investigators have used extraction from ground tissue with physiological saline (*Rosenmann* 1920, 1923, 1936, *Huggins, Vail* and *Davis* 1943, *Macfarlane* and *Biggs* 1948, *Kaulla* and *Shettles* 1954, *Fantl* and *Fitzpatrick* 1950, *Astrup* and *Sterndorff* 1952 a), with distilled water (*Frankl* and *Aschner* 1911, *Page, Glendening* and *Parkinson* 1951) or with phosphate buffer in saline (*Tagnon, Schulman, Whitmore* and *Leone* 1953, *Tagnon, Whitmore, Schul-*

man and *Kravitz* 1953, *Tagnon* 1954). The ability of these extracts to digest various fibrin substrates gave a measure of the fibrinolytic activity of the tissues. However, various investigations have shown that the tissue activator cannot be extracted from the tissues without the use of specific solvents, because it is firmly bound to the tissue proteins (*Fleisher* and *Loeb* 1915, *Permin* 1947, *Astrup* and *Stage* 1952, and others). A quantitative determination is therefore not possible by these methods of extraction. Several of the methods were not qualitative either, since the tissue extracts were measured against plasminogen-containing substrates.

Other methods have been described by *Greenberg* (1948), who measured the ability of ground and freeze-dried tissues to lyse fibrin substrates by determining the time required for lysis, and by *Bierstedt* (1955 a, b, c), who extracted the tissues with a solution of magnesium sulphate and sodium chloride after having shown that the presence of magnesium ions rendered the active substances in the tissues soluble. The time required for the lysis of a mixture of this tissue extract and human fibrin gave an expression of the content of active substances in the extract. However, owing to the plasminogen content of the fibrin substrates these methods cannot be used for qualitative analyses either.

After the discovery of the tissue activator of plasminogen (*Astrup* and *Permin* 1947), other investigators elaborated methods for the determination of its content in various tissues. *Tagnon* and *Petermann* (1949 a, b) and *Tagnon* and *Palade* (1950) thus demonstrated a plasminogen activator in the rat lung. The tissue was ground, suspended in a 30 % glucose solution and subjected to differential centrifugation in the cold. To the various fractions was then added a mixture of 0.2 % fibrinogen solution in phosphate buffer and human plasminogen (prepared by precipitation with ammonium sulphate at pH 7, redissolution of the precipitate in saline, and dialysis). After addition of thrombin the time required for lysis was determined. When the tissue fraction was omitted, no fibrinolysis occurred, and the same observation was made, although less constantly, when plasminogen was left out. It was thus shown that both the tissue fraction and plasminogen must be present in order to effect fibrinolysis. The greatest activity was found in the microsome fraction. In a few cases, lysis occurred in the absence of plasminogen,

which was explained by the presence of this factor in the fibrin. Similar methods were used by *Lewis* and *Ferguson* (1950) and *Philips, Butler* and *Taylor* (1956). *Fantl* and *Fitzpatrick* (1950) observed fibrinolytic activity in a mixture of oxalated plasma, brain extract (prepared from acetone-dried brain tissue by extraction with 0.85 % saline) and calcium chloride, whereas fibrinolysis did not occur when the plasma was clotted with calcium chloride and the serum removed by centrifugation before the brain extract was added. A substance in the brain extract in combination with a factor in the serum was thus responsible for the fibrinolytic activity. In a few cases, fibrinolytic activity was nevertheless observed although the serum had been removed before the addition of the tissue extract, which was explained by the presence of plasminogen in the fibrin. *Permin* shook various tissue fragments for 24 hours at 39° C. with (1) physiological saline, (2) fibrinogen solutions and (3) solutions prepared from (2) by removal of the fibrinogen either by clotting with thrombin or by heating. After centrifugation, the supernatant fluid was removed and placed on a thin layer of bovine fibrin on the bottom of a Petri dish. It was shown that the saline extract did not exert any effect on the fibrin, while the other extracts were fibrinolytically active. It was thus demonstrated that a factor in the tissue in combination with one in the fibrinogen solution may form a fibrinolytic enzyme, and that the latter factor is not attached to the fibrinogen itself. In order to confirm this observation, *Permin* performed some experiments with casein. Casein does not contain plasminogen and therefore cannot be broken down by the tissue activator of plasminogen. On the other hand, if plasminogen is added to the casein, a decomposition occurs when acetone-ether-dried tissue from the pig heart is brought into contact with the casein, evidencing that the tissue activator present in the heart tissue has converted the plasminogen into plasmin. Similar methods with acetone-dried tissue have later been used by *Astrup* (1951 a, b). *Astrup* and *Sterndorff* (1952 a) found that saline extracts from certain tissues are able to lyse bovine fibrin, but not bovine fibrin which had been heated to 85° C. for 35 minutes. As this heating destroys the plasminogen in bovine fibrin without a corresponding destruction of the fibrin (*Lassen* 1952), these results show that the tissue extracts concerned contain plasminogen activator. It has later

been shown that extraction with potassium thiocyanate results in the formation of a solution which is more active than saline extracts from the same tissues, and that this potassium-thiocyanate extract has no effect on heat-treated fibrin (*Astrup* and *Stage* 1952). This was later confirmed by *Albrechtsen* (1958). Potassium thiocyanate is thus a specific solvent for the tissue activator of plasminogen. Analyses of potassium-thiocyanate extracts from certain tissues by means of the fibrin-plate method have later been performed by *Astrup* and *Sterndorff* (1956 a).

The aforementioned methods are useful for the specific determination of tissue activators of plasminogen, but do not give any quantitative expression of the content of the tissue activators. It was therefore necessary to elaborate such a quantitative method (*Astrup* and *Albrechtsen* 1957). This method is based on the observation that potassium thiocyanate is a specific solvent for the tissue activator of plasminogen (*Astrup* and *Stage* 1952). It was shown that by repeated extraction with a 2-M potassium-thiocyanate solution it is possible to extract the tissue activator quantitatively from a tissue sample in a soluble form. In addition to the tissue activator, such an extract may contain other fibrinolytic components from the tissue (for example, tissue inhibitors, fibrinolytic components from the blood or other body fluids present in the tissue and, possibly, proteolytic enzymes (cathepsins)). However, by treatment with hydrochloric acid at pH 1 it is possible to precipitate the tissue activator from such a solution, in this way separating it from tissue inhibitors of the pulmin type; at the same time, this precipitation at an acid reaction destroys the labile fibrinolytic components present in the blood. The precipitated tissue activator is stable at acid reaction (*Astrup* and *Sterndorff* 1956 a) and may be redissolved in 2-M potassium thiocyanate. The solution prepared in this way therefore contains the total amount of tissue activator from a given tissue sample, and the activity in the solution will not be inhibited or stimulated by other fibrinolytic components. It has further been shown that the presence of potassium thiocyanate in the solution does not influence the fibrinolytic activity. The precipitation at acid reaction also renders it possible to separate the tissue activator and the inhibitor present in human urine, a possibility which is of importance in the determination of the amount of tissue activator

in the kidney. The content of tissue activator in the solution is expressed in terms of its ability to digest bovine fibrin (fibrin-plate method). However, if such an analysis is to be quantitative, it must be required that the composition of the bovine fibrin used for the fibrin plates is constant from one determination to another, but this is not the case, as the bovine plasma used as the fibrin source obviously originates from different animals. It appears that a given amount of tissue activator shows variable activity against different fibrin substrates, even when these have been prepared in the same way. This phenomenon may be partially explained by the fact that fibrinolytic inhibitors are present in variable amounts in bovine fibrinogen from different animals. Thus, as the fibrin cannot be used as a standard substrate in the determination of the tissue activator, it was necessary to prepare a standard powder of a tissue activator, and then express the activity observed in the extracts as percentages of this standard.

In practice, certain difficulties were involved in preparing such a tissue-activator standard. The standard used in the method was prepared from washed and acetone-dried pig-heart tissue (*Astrup* 1951 a). After extraction with 2-M potassium thiocyanate, precipitation with hydrochloric acid at pH 1, redissolution of the precipitate in a saline buffer and freeze-drying, a white, freely soluble powder with considerable activity was obtained. Stored in stoppered bottles at –20° C., this powder remained stable for at least 18 months. However, very careful storage of the powder is required if the activity is to be preserved. It is hygroscopic and loses some of its activity under the influence of water. One unit of tissue activator is defined as 1 mg of this standard powder. The content of tissue activator in the analysed tissue sample can be expressed as a percentage of the standard by applying various dilutions of both the tissue extract and the standard solution to fibrin plates and plotting the results read in a double logarithmic graph in which the logarithm of the concentration is the abscissa and the logarithm of the area of the lysed zone is the ordinate.

Later experiments have shown that the practical difficulties involved in the preparation and storage of this standard powder are so great that it is doubtful if it can be used for routine determinations over a number of years, especially in laboratories which do not

constantly deal with fibrinolytic problems. In order to obtain a more stable product, it has therefore been contemplated to prepare a standard powder directly by grinding of acetone-dried pig-heart tissue.

The method described renders it possible to determine the content not only of tissue activator but also of tissue inhibitors of the pulmin type, since the latter, as already mentioned, are present in solution after the acid precipitation used. They can be precipitated from this solution with sodium wolframate and be measured by their inhibitory action on trypsin.

Properties of the Tissue Activator

It has proved difficult to analyse the properties of the tissue activator, because it has not yet been possible to purify it. However, it marks a great step forward that it may now be prepared in a soluble form, since the amount of impurities in such a solution can be considerably reduced.

Thermostability.—Whereas the fibrinolytic activity of the tissues is not diminished by heating to 29 and 37° C. at neutral reaction (*Lewis* and *Ferguson* 1950, *Tagnon* and *Petermann* 1949 b), higher temperatures exert a more or less destructive action on the activity, depending on the period of heating. *Fleisher* and *Loeb* (1915) observed a diminished activity after heating to 49 and 56° C. for 30 minutes, a finding which has later been partially confirmed by *Permin* (1947), *Tagnon* and *Petermann* (1949 b) and *Lewis* and *Ferguson* (1950). Still higher temperatures (70–80° C.) result in rapid destruction (*Caffier* 1930, *Permin* 1947, *Fantl* and *Fitzpatrick* 1950, *Lewis* and *Ferguson* 1950). On the other hand, low temperatures do not seem to destroy the activity of the tissues. The tissue activator may thus be stored in a frozen state without losing its activity (*Tagnon* and *Petermann* 1949 b, *Lewis* and *Ferguson* 1950, *Astrup* 1951 a), and at the freezing point loss in the activity occurs only after storage for a long time. Detailed studies on solutions of the tissue activator (*Astrup* and *Sterndorff* 1956 a) have shown that it possesses great stability at neutral reaction after heating to 37° C. for 30 minutes and less stability at 50 and 70° C., and that

it is destroyed by heating to 100° C. These results have later been confirmed by *Albrechtsen* (1956 a, 1958).

Stability at various pH levels.—The tissue activator is influenced by changes in the hydrogen-ion concentration. *Demuth* and *v. Riesen* (1928 a) observed that the fibrinolytic activity of tissues was greatest at acid reaction. This was partially confirmed by *Permin* (1947), who found greater stability at neutral and weakly acid pH levels and destruction of the tissue activator at alkalinity. The reaction optimum was found to be at pH 6 (*Permin* 1949). Similar observations were made by *Tagnon* and *Petermann* (1949 b) (reaction optimum within the range from pH 0 to 7.2), *Permin* (1950) and *Tagnon* and *Palade* (1950). More recent studies on a saline-soluble tissue activator derived from pig hearts (*Astrup* and *Sterndorff* 1956 a) revealed stability on heating to 37° C. for 30 minutes within a very wide range of pH values, viz. from pH 1 to about pH 8. On heating to 50° C., the greatest activity was found at acid reaction and a minor fall at neutral and alkaline reaction, while heating to still higher temperatures (70 and 100° C.) gave stability at markedly acid reaction and partial or complete destruction at more alkaline levels. These observations were later confirmed by *Albrechtsen* (1956 a, 1958). The tissue activator is thus relatively stable, especially at acid pH levels and differs in that respect from the labile plasminogen activator which is present or can be formed in blood and certain secretions and transudates (*Müllertz* 1955 a, 1956, *Storm* 1955, *Albrechtsen* and *Thaysen* 1955, *Albrechtsen* and *Trolle* 1955, *Albrechtsen, Storm* and *Claassen* 1958).

Stability to chemicals.—The tissue activator is stable against the influence of a number of chemicals. Thus, acids, bases, toluol, alcohol, ether and chloroform do not affect the fibrinolytic activity of the tissues (*Fleisher* and *Loeb* 1915); nor is it influenced by acetone (*Permin* 1947). On the other hand, formalin destroys the active components in the tissue (*Caffier* 1930). This pronounced chemical resistance has been confirmed by several investigators (*Astrup* and *Permin* 1947, *Permin* 1949, 1950, *Astrup* 1951 a, *Astrup* and *Sterndorff* 1956 a). In the elaboration of measuring methods it has been of importance to ascertain that neither potassium thiocyanate (*Astrup* and *Albrechtsen* 1957) nor magnesium sulphate (*Bierstedt* 1955 c) destroys the tissue activator.

Binding of the tissue activator to tissue proteins.—The tissue activator is firmly bound to the tissue proteins and can be extracted only by specific solvents. As already mentioned, this has caused considerable difficulties in the attempts to elaborate methods for its quantitative estimation. It was observed long ago that saline was an unsuitable solvent (*Halban* and *Frankl* 1910). Although this has later been confirmed in some experiments (*Fleisher* and *Loeb* 1915), others have shown that it is, in some cases, possible to prepare active saline extracts from certain tissues (*Macfarlane* and *Biggs* 1948, *Fantl* and *Fitzpatrick* 1950, *Tagnon* 1954, *Astrup* and *Sterndorff* 1952 a). It has, in particular, proved to be easy to extract the tissue activator from brain tissue, possibly because the presence of phospholipids increases the solubility of the tissue activator (*Fantl* and *Fitzpatrick* 1950). However, the tissue activator cannot be extracted quantitatively by means of saline (*Astrup* and *Stage* 1952, *Albrechtsen* 1958), and a number of other solvents, such as phosphate buffer, acetic acid, hydrochloric acid, ammonium hydroxide and glycerin, have also proved to be unsuited for quantitative extraction (*Permin* 1947, 1950, *Lewis* and *Ferguson* 1950, *Loomis* 1950). On the other hand, potassium thiocyanate is a specific solvent (*Astrup* and *Stage* 1952), and the presence of magnesium ions increases the solubility of the tissue activator (*Bierstedt* 1955 a, b, c). The tissue activator must be assumed to be firmly bound to the tissue proteins and is, according to *Tagnon* and *Petermann* (1949 a, b), *Lewis* and *Ferguson* (1950) and *Tagnon* and *Palade* (1950), present mainly in the microsomes.

Activation of Plasminogen by the Tissue Activator

The transformation of plasminogen into plasmin under the influence of the tissue activator has been the subject of several studies. It has been shown (*Astrup* 1951 a, b, 1952 b) that the amount of plasmin formed by this process varies with the concentrations of both the tissue activator and plasminogen in such a manner that increasing amounts of plasmin are formed on addition either of increasing amounts of tissue activator to the same amount of plasminogen or of increasing amounts of plasminogen to the same amount of tissue activator. The reaction proceeds towards equilib-

rium within a given time, so that complete conversion of the plasminogen in a given solution cannot be obtained even on addition of the strongest concentrations of tissue activator. It has further been shown that the tissue activator inhibits the plasmin produced. On the basis of these observations the theory has been advanced that the tissue activator reversibly removes a blocking substance from the plasminogen, so that this is converted into plasmin. Accordingly, the blocking substance should be attached to the tissue activator, and this complex should be able to inhibit the plasmin produced. Both *Permin* (1949) and *Lewis* and *Ferguson* (1950) confirmed that the amount of plasmin formed by this process depends on the amounts of both plasminogen and tissue activator. Moreover, *Permin* showed that the process is at a maximum between 20 and 39° C. and at a minimum at 0° C., and is roughly independent of the pH level within the range from 4 to 10. However, in later investigations, *Tagnon* and *Petermann* (1949 b) and *Tagnon* and *Palade* (1950) were unable to confirm these observations. They found that formation of increasing amounts of plasmin occurred only on addition of increasing quantities of tissue activator to a given amount of plasminogen, whereas, conversely, addition of increasing quantities of plasminogen to a given quantity of tissue activator did not increase the amount of plasmin produced. They also found that the process had its reaction optimum at pH 6.0–7.2 at 37° C.

Thus, the conditions are not at all clarified, and it seems as if the problem cannot be solved until it is possible to obtain pure preparations for the experiments and to use better methods for the estimation of the reactions (*Astrup* 1956 d), since, for example, the presence of inhibitors in the solutions must be assumed to exert a great influence on the progress of the reaction.

Fibrinolytic Activity in Animal Tissues

A number of experiments have shown that the fibrinolytic activity in animal organs varies from species to species and from organ to organ in the same animal. As early as 1915, *Fleisher* and *Loeb* revealed wide variations in the fibrinolytic activity of various tissues from the guinea pig, rabbit, rat, cat, mouse and chicken on

plasma from the rabbit, chicken and guinea pig. However, a certain conformity was observed, since the activity was present in the same types of tissues (intestine, ovary, nervous system, kidney, uterus, and others). Hepatic tissue and connective tissue did not show any fibrinolytic activity. The fibrinolytic activity was not particularly directed against homologous plasma. Subsequent studies have revealed fibrinolytic activity in tissues from the ox and pig (spleen, lung, liver, kidney, thyroid gland and heart), evidenced by the fact that dialysed fluid expressed from these tissues were able to digest fibrin (*Rosenmann* 1923, 1936). The qualitative shortcomings in the above-mentioned methods and in a large number of tissue-culture experiments (for references, see *Santesson* 1935 and *Permin* 1949) render it difficult to assess the value of the experiments.

Specific methods for the determination of the tissue activator in animal organs have revealed variations from organ to organ in the same animal (ox, pig, rabbit and rat) and between organs from different animals (*Astrup* and *Permin* 1947). This has later been confirmed by *Permin* (1947, 1949, 1950), who found the greatest activity in organs from calf embryos, pigs and rabbits, while organs from the ox, rat and mouse were less active and those from hens and chickens were practically inactive. In particular, pulmonary tissue was greatly active (the ox and pig were not studied), while the liver and spleen were inactive. Later investigations have confirmed the presence of large amounts of plasminogen activator in pulmonary tissue from various animal species (rat, dog, mouse, guinea pig and cat) (*Tagnon* and *Petermann* 1949 a, b, *Tagnon* and *Palade* 1950, *Lewis* and *Ferguson* 1950). Plasminogen activators have also been found in the rabbit brain (*Fantl* and *Fitzpatrick* 1950), in the pancreas, gall bladder, ovary and lymph nodes of the dog (*Lewis* and *Ferguson*) and in brain tissue from the dog and rabbit (*Loomis* 1950). Several investigations performed by *Astrup* and his co-workers revealed the presence of a plasminogen activator in the heart, brain and lung of the pig, while the ox lung was found to be inactive.

The above-mentioned qualitative analyses have been of value because they have given information as to the types of tissue in which the tissue activator is present, but owing to the quantitative shortcomings of the methods the results obtained are not directly

Table 1. Content of tissue activator in various animal organs, expressed in tissue-activator units pe

Organ	Pig			Horse			Ox			Dog			Ra
Brain.....	69	32	32	130	69	22	75	75	54	11	11	5	+
Lung.....	0	0	0	97	45	43	0	0	0	23	12	8	0
Heart.....	216	216	216	45	25	25	34	17	34	0	0	0	0
Uterus.....	313	76	47	180	126	108	9	0	0	10	58	54	40
Muscles.....	50	22	0	108	108	38	0	0	0	0	0	0	0
Prostate.....	5	5		238	65	180	0	0	11	216	108	76	0
Adrenal.....	97	37	32	30	17	8	65	34	15	37	37	24	0
Testis.....	0	0		32			11	11	14	19	11	6	0
Ovary.....	292	130	97	36	25	22	23	8	6	25	54	0	27

comparable. A renewed analysis using a quantitative method (*Astrup* and *Albrechtsen* 1957) was therefore necessary (*Albrechtsen* 1957 b). This analysis confirmed that the content of tissue activator varies from organ to organ in the same animal, from animal to animal of the same species, and from species to species. A variety of tissues were analysed (brain, lung, heart, uterus, skeletal muscles, prostate, adrenal, testis, ovary, kidney, liver and spleen) from a number of animals (pig, horse, ox, dog, rabbit, guinea pig, cat, mouse, rat, codfish, fowl and calf embryos). The following results were obtained (table 1):—

Brain tissue is active in most species except the cat, but the activity of the rabbit brain is very slight.

Pulmonary tissue is likewise active, except in the pig, ox and rabbit. Pulmonary tissue from the mouse and rat shows considerable activity. The finding that activity is absent in pulmonary tissue from the pig is not in agreement with previous observations. Thus, *Astrup* (1952 a) and *Astrup* and *Sterndorff* (1956 a) found that the pig lung contained considerable amounts of plasminogen activator, and in some preliminary experiments preceding the investigations

ssue-activa its per gramme of fresh tissue. The three values refer to three different samples.

Dog		Rabbit			Guinea pig			Cat			Mouse			Rat		
11	5	+	0		25	29	18	0	0	0	13	+	+	19	27	29
12	8	0	0		14	18	12	25	16	0	238	216	184	248	245	203
0	0	0	0		32	50	36	0	0	0	0	0	0	19	19	23
58	54	40	40		68	32	36	14	27	24	+	+	7	14	12	5
0	0	0	0		0	0	0	0	0	0	0	0	0	0	+	0
108	76	0	0													
37	24	0	0		8	5	6	36	17	7				77	58	45
11	6	0	0		31	9	5	0	0	0						
54	8	27	8					+	10	7				99	54	29

of *Albrechtsen* (1957 b) these observations were confirmed. So far this question remains unexplained.

The pig heart is very active, whereas the activity of heart tissue from the horse, ox, guinea pig and rat is moderate, and the heart of the dog, rabbit, cat and mouse is inactive.

The uterus from all these animals is active.

The skeletal muscles is inactive except in the pig and horse.

The prostate from all the animals examined (with the exception of the rabbit) contains tissue activator, in the horse and dog in considerable concentrations.

The adrenals are also active in all the animals except the rabbit, and the ovary is active in all of them.

On the other hand, the testis is inactive in the pig, rabbit and cat, but active in the other animals studied.

In most of the animals, the kidney is either inactive or only slightly active.

The liver and spleen contains no tissue activator.

Organs from codfish and fowl are inactive. The study also confirmed the observation made by *Permin*, viz. that the concentration

Table 2.

Content of tissue activator in organs from calf embryos, calves and adult oxen, expressed in tissue-activator units per gramme of fresh tissue.
+ i. indicates the presence of trypsin inhibitors of the pulmin type.

Length	Heart	Lung	Kidney	Spleen	Liver	Muscles	Brain	Adrenal.	Testis
7 cm		32			0		19		
9 cm	47	17			0		20		
20 cm	68	32	34	0	45	0	0		
30 cm	81	9	14	0	14	0	7		
36 cm	61	14	17	14	0	0	0		
50 cm	68	19	23	19	3	3	7	68	
58 cm	79	45	30	13	18	6	18	146	
65 cm	27	19	14	9	0			28	8
70 cm	34	23	23	17	5	8			
85 cm	39	14	39	7	1	2		72	
90 cm	42	27	10	7	0		6		16
132 cm	72	9+i.	34	9	0	0	26	63	
145 cm	5	5	7	3	0	0	5	23	
155 cm	42	0+i.	28	12	0	0	34	34	
Calf...	28	14+i.	20	7	0				14
Calf...		6+i.							
Calf...	39	0+i.	12	3	0	0		30	0
Calf...		0+i.							
Calf...		0+i.							
Adult ox	17	0+i.	0	0	0	0	75	65	11
Adult ox	34	0+i.	0	0	0	0	75	34	11
Adult ox	34	0+i.	0	0	0	0	54	15	14

of tissue activator is higher in calf embryonic tissues than in tissues from the adult ox. This applies to the lung, kidney, spleen, liver and skeletal muscles, whereas the same concentration of tissue activator is found in the heart, brain, adrenal and testis of the embryo and the adult animal. The tissue activator disappears from the lung, kidney, spleen, liver and skeletal muscles shortly after birth (table 2).

In a series of studies on the presence of tissue activator in the central nervous system, *Moltke* (1957) found considerable concentrations in both the dura mater and pia mater in the monkey, horse, ox and pig. Finally, *Roberts* and *Astrup* (1957) investigated the conditions as to the concentration of tissue activator in organs from Rhesus and Java monkeys. Their investigations confirmed that the

Table 3.

Content of tissue activator in organs from Rhesus and Java monkeys, expressed in tissue-activator units per gramme of fresh tissue (after *Roberts* and *Astrup* 1957).

Type of tissue	No. of Samples	Units per gramme of tissue		
		Maximum	Minimum	Average
Periosteum	5	438	95	322
Meninges	2	381	184	283
Epididymis	3	190	133	167
Prostate	1	161	—	161
Trachea	8	230	58	145
Uterus	4	334	6	100
Caecal mucosa	4	161	5	85
Vagina	2	115	16	65
Skeletal muscles	8	121	11	58
Brain	3	70	37	50
Thymus	2	46	30	38
Lymph nodes	2	61	8	35
Heart muscle	6	72	4	31
Testes	2	33	25	29
Skin	7	69	2	28
Bladder epithelium	6	45	0	25
Ovaries	4	95	6	25
Smooth muscles (oesophagus, stomach, intestine)	11	60	0	24
Intestinal epithelium (oesophagus, stomach, small intestine)	13	72	0	20
Lung	6	54	0	17
Spleen	6	5	0	1
Cartilage	5	3	0	1
Tendon	1	2	0	2
Liver	7	0	0	0
Bone	4	0	0	0

concentration varies considerably from animal to animal and from organ to organ in the same animal. The highest concentrations were found in the periosteum, meninges, epididymis, prostate, trachea and uterus, while osseous, hepatic and tendon tissues were largely inactive (table 3).

Fibrinolytic Activity in Human Tissues

Only a limited number of studies on fibrinolytic activity in human tissues are available. This is surprising since the tissue

Table 4. Content of tissue activator in human organs (autopsies)

Sex	Age	Uterus	Adrenal	Lymph node	Prostate	Thyroid	Lung	Ova
M.....	0		63		81			
F.....	3	720	576				648	
F.....	5		360			189	297	
F.....	11	450	333	1278			243	40
F.....	18	540	270	540			450	135
M.....	22		450		405	207	279	
M.....	32		90	270	414		72	
M.....	32		360	16	279		104	
F.....	42					234		
F.....	44	900	234	540			225	378
M.....	46		315		630	504	90	
M.....	50		414	99		198	189	
M.....	52		1278		432		378	
F.....	56						41	135
M.....	59					414		
M.....	64		450	180	306		180	
F.....	64	810	514				108	364
M.....	66							
M.....	68					297		
M.....	74					324		
F.....	74					432		
M.....	77		450	99	126		38	
F.....	80	900						
M.....	82					450		
Mean.....		720	410	378	334	325	223	210

activator must be considered to be of significance in the activation of fibrinolysis in vivo. *Huggins, Vail* and *Davis* (1943) demonstrated fibrinolytic activity in saline extracts from the prostate and thyroid gland, and the first systematic investigations on the fibrinolytic activity of human tissues (*Macfarlane* and *Biggs* 1948) showed wide variations from organ to organ. The fibrinolytic activity was expressed by the ability of saline extracts to lyse human fibrin. The adrenal, lung and kidney were highly active, while the thyroid gland and brain were moderately active, and the activity of the skeletal muscles, heart, liver and spleen was very slight. In early studies, *Rosenmann* (1920) demonstrated fibrinolytic activity in the human lung, an activity which has later been shown to be referable to a

rgans (autopsies), expressed in tissue-activator units per gramme of tissue.

id	Lung	Ovary	Pituitary	Kidney	Muscle	Heart	Brain	Testis	Spleen	Liver
				144					18	0
	648			144	324	36	20		0	0
	297		77	342	113	113	8		0	0
	243	40		54	54		23		19	
	450	135		81	180	58	38	0		0
	279		92	81	32	64	38	9	0	0
	72			19	216	41	54	23		
	104			144	171	117	43			
	225	378		90	153	144			5	0
	90		144	0	14	77	65	68	0	0
	189		113	126	59	95	20	0	99	0
	378			252	95	126		15		0
	41	135		16	27	27				0
	180			50	126	81		63		
	108	364		148	180	86			41	
							38			
			126							
			288							
	38			215		77		19		6
	223	210	140	119	110	82	35	25	20	0

plasminogen activator (*Permin* 1950, *Lewis* and *Ferguson* 1950, *Astrup* and *Sterndorff* 1952 a). Plasminogen activators have also been found in human brain tissue (*Permin* 1950, *Fantl* and *Fitzpatrick* 1950), and in the heart, skeletal muscles and kidney, whereas no activators could be demonstrated in the liver and spleen. Finally, *Bierstedt* (1955 a, b, c) revealed fibrinolytic activity in magnesium-sulphate extracts from various human tissues, of which muscular organs, such as the oesophagus and bladder, were highly active.

As previously pointed out, the methods used in all the aforementioned studies had certain qualitative or quantitative shortcomings, for which reason renewed studies on this problem were required. Using a quantitative and selective method for the determina-

tion of the concentration of tissue activator in freshly removed organs (*Astrup* and *Albrechtsen* 1957), studies were performed by *Albrechtsen* (1957 a). In an analysis of 155 different organs from 24 cadavers, it was shown that the concentration of the tissue activator (expressed in units per gramme of fresh tissue) varies considerably from individual to individual and from organ to organ in the same individual. In spite of these wide variations, it was possible to divide the organs into three groups with high, moderate and low contents of the tissue activator (table 4).

The first group (with an average content of more than 200 tissue-activator units per gramme of tissue) comprises the adrenal, prostate, uterus, lymph nodes, thyroid gland, lung and ovary. The second group (25 to 200 units per gramme of tissue) consists of the pituitary body, kidney, skeletal muscles, heart, brain and testis, while only very slight activity was found in a few samples of the spleen and no activity at all in samples from the liver. No correlation seemed to exist between the concentration of the tissue activator and the sex or age of the individual, and the activator did not seem to be localised in certain types of tissue. The almost complete absence of activity in the liver was a characteristic feature, in harmony with previous observations. Recent studies by *Moltke* (1957) have shown that the concentration of the tissue activator is relatively high in the pia mater and somewhat less in the dura mater.

Significance of the Tissue Activator in Physiological and Pathological Processes

The physiological significance of the tissue activator is still unknown. The investigators who have been concerned with analyses of the activator have also advanced certain hypothetical considerations on its functions in order to throw light on its importance in the organism. It is reasonable to assume that the tissue activator of plasminogen, under certain conditions, must be able to influence the plasminogen in the fibrin deposits which may be formed in the organism. Thus, *Fleisher* and *Loeb* (1915) and *Rosenmann* (1920) suggested that the fibrinolytic activity of the tissues might prevent the formation of connective tissue, since a release of this activity through a digestion of fibrin might destroy the growth substrate of

the connective-tissue cells and thus prevent growth. Any more or less extensive injury to tissues of the organism is likely to give rise to the formation of fibrin clots, presumably through a release of thromboplastin from the tissues. If such fibrin formations persist, ingrowth of fibroblasts from resting connective-tissue cells will occur within a short space of time, resulting in the formation of collagen fibrils. Connective tissue is then formed (*Astrup* 1956 b). Even under normal conditions, the frequent occurrence of minute injuries may give rise to such processes. If the formation of this connective tissue is to be avoided, the fibrin deposits produced must be removed. In the organism, several processes are available for this purpose. Liberation of cathepsins from the injured tissues or of proteases from dead leukocytes (infected wounds) may be active in this respect. However, the hypothesis has been advanced that the fibrinolytic enzyme system is a very powerful factor in the restoration of normal conditions in traumatised tissue (*Astrup* 1955 a, 1956 a, b, *Astrup* and *Sterndorff* 1956 a, *Albrechtsen* 1957 a). It is likely that the activation occurring under these conditions is caused directly by the tissue activator of plasminogen. However, fibrinolytic activation is possible only when the tissue activator comes into contact with the plasminogen in the fibrin deposits, and certain conditions must be present before this occurs, since the tissue activator is firmly bound to the tissue proteins. Tissue damage and the resultant cell necrosis are presumably important factors in this connexion. Thus, it has been shown experimentally that during the course of cell necrosis the tissue activator is released from the cells, causing fibrinolytic activity outside the tissue (in the human endometrium during menstruation (*Albrechtsen* 1956 a, b)), and it would therefore be reasonable to assume that a similar process may occur in other organs containing the tissue activator.

The interaction between the clot-promoting and fibrinolytic properties of the tissues must be closely regulated. Thus, diminished fibrinolytic activity might be conceived to result in the formation of connective tissue, while, conversely, an increased fibrinolytic effect, through a too rapid removal of the haemostatic fibrin deposits, might give rise to continued bleedings in the tissues. It has therefore been important to determine the distribution of fibrinolytic activity in the various tissues. However, the processes are presumably

not quite so simple as suggested here, since, for example, the fibrinolytic tissue inhibitors must be assumed to exert an influence on the progress of the reactions.

The relation between the tissue activator and the fibrinolytic activity, which has been observed in the circulating blood under both physiological and pathological conditions, is as yet unknown. Several facts weigh against the assumption that the tissue activator can be released from the tissues into the blood stream and thus bring about a conversion of plasminogen into plasmin. In this connexion, the powerful binding to the tissue proteins must, above all, be considered. The tissue activator has therefore been regarded as a component with an exclusively local action in the organism (*Astrup* 1955 a, 1956 a, b, d). It has further been shown that the plasminogen activator which is present in the blood (*Müllertz* 1955 c, 1956) is not identical with the stable tissue activator, since it is relatively labile, especially at acid reaction. The formation of plasminogen activators must therefore be supposed to occur through a different mechanism, viz. by transformation of the plasminogen pro-activator under the influence of so-called lysokinases (*Müllertz* and *Lassen* 1953, *Astrup* 1955 a, 1956 a). However, the presence of such lysokinases in the organism have not yet been unquestionably demonstrated, although some investigations have revealed signs of lysokinase activity in certain human tissues (*Astrup* and *Sterndorff* 1956 b) and in the blood (*Müllertz* 1955 a, b).

While the aforementioned observations thus seem to speak against the assumption that the tissue activator is of significance in the fibrinolytic activity of the circulating blood, other facts are in favour of such an assumption. It thus appears that although the tissue activator is firmly bound to the tissues, it is possible, simply by means of physiological saline, to extract plasminogen activators, in some cases even in considerable amounts, from the tissues which are most active (*Albrechtsen* 1958). It must therefore be concluded that part of the tissue activator of plasminogen is less firmly bound to the tissue proteins. The plasminogen activator extracted by saline is somewhat reminiscent of the plasminogen activator in blood with regard to its stability at various temperatures and pH levels. It is therefore possible that the tissue activity may in certain cases be the cause of fibrinolysis in the circulating blood. Although resting on a

more slender foundation, such a theory has, in fact, been advanced by *Caffier* (1930), *Rosenmann* (1936), *Mirsky* and *Freis* (1944) and *Mole* (1948). Moreover, since, according to *Stefanini* (1952), the most frequent cause of fibrinolytic activity in the blood is tissue injuries, this fibrinolysis has been regarded as being due to liberation of plasminogen activators from the damaged tissues (*Tagnon* 1953). In a number of studies, it has been shown that tissue injuries in certain operations are frequently combined with fibrinolytic activity in the blood, and that this activity has occasionally given rise to haemorrhagic diathesis. This has particularly been observed in operations on the lung (*Mathey, Daumet, Soulier, Bolloch* and *Fayet* 1950, *Soulier, Mathey, Bolloch, Daumet* and *Fayet* 1952, *Chalnot, Michon* and *Lochard* 1952, *Soulier* 1952, *Baumann* 1952, *Coon* and *Hodgson* 1952, *Penn* and *Walker* 1954, *Blombäck, Blombäck, Senning* and *Wallen* 1955, and others). The theory of a release of fibrinolytic activators from the lung under these conditions is supported by the observation that the blood from the pulmonary vein shows a higher fibrinolytic activity than that from the pulmonary artery (*Mathey* et al.). Similar observations have been made in operations on the thyroid gland (*Lhoiry* and *Fayet* 1954) and, as will be mentioned later, on the prostate and uterus; all these organs have a high content of tissue activator. These questions have been considered in reviews by *Stefanini* and *Dameshek* (1955) and *Astrup* and *Albrechtsen* (1956), in which it is also mentioned that injuries to tissues containing the tissue activator either by burns or due to malignant disease may also be associated with fibrinolytic activity in the blood and by haemorrhagic diathesis.

Fibrinolytic activity of the blood has also been observed post mortem (for references, see, for example, *Mole* 1948), in which case the activity varies with the cause of death (*Müllertz* 1952). This activity is responsible for the incoagulability of post-mortem blood. It has later been shown that this fibrinolysis is due to a plasminogen activator (*Müllertz* 1953). Opinions are also rather divergent as to the mechanism of activation post mortem. *Mole* (1948) assumed that a release of activating agents from the endothelium was responsible for post-mortem fibrinolysis, but this assumption has not been confirmed. Later investigations (*Astrup* and *Claassen* 1957) have shown that tissue activators are absent both in the intima and tunica

media, whereas they are present in a high concentration in the adventitia. *Astrup* (1952 b) and *Müllertz* (1952) assumed that the fibrinolysis was referable to a liberation of tissue activators brought about by an increased permeability of the cells. *Bierstedt* (1955 a, b, c) studied the relation between the fibrinolytic activity of tissues and the activity in blood from human cadavers. He found that a high fibrinolytic activity in the tissues was accompanied by a high activity in the venous blood, while arterial blood showed less fibrinolytic activity. Accordingly, he concluded that the fibrinolytic activity of the blood originated from the tissues. However, *Müllertz* (1953) found that the plasminogen activator in post-mortem blood was more labile than the tissue activator of plasminogen, and therefore concluded that this plasminogen activator in the blood was formed from a precursor in the blood and did not originate from the tissues. The problem is thus analogous with that involved in the mechanism of activation in vivo, but neither of them has so far been clarified.

Chapter II

FIBRINOLYTIC ACTIVITY OF THE UTERUS

Introduction

The fibrinolytic activity of the myometrium, endometrium and of menstrual blood has been the subject of several studies. We are here facing fibrinolytic processes which occur in an anatomically well-defined region. By a closer analysis of these processes it would be possible, first, to solve the local problems concerning the incoagulability of menstrual blood and the shedding of endometrial tissue during the menstruation and, secondly, by the solution of these local problems, to contribute to a better understanding of the fibrinolytic processes in other parts of the organism.

Fibrinolytic Activity of the Myometrium

Like several other types of tissue, that of the myometrium is capable of digesting fibrin. This was demonstrated as early as 1915 by *Fleisher* and *Loeb,* who studied the uterus from various species (guinea pig, rabbit and rat). They concluded that a fibrinolytic ferment in the myometrium (fibrinolysin) caused this fibrinolysis. Similar fibrinolytic properties have later been demonstrated in the myometrium of dogs (*Hamilton, Higgins, Mills, Lawrence* and *Helwig* 1950), rabbits (*Page, Glendening* and *Parkinson* 1951) and man (*Greenberg* 1948, *Bierstedt* 1955 c). These investigations were performed on untreated or extracted myometrial tissue and plasminogen-containing fibrin substrates, for which reason the nature of the active components remained unknown. A more detailed knowledge was obtained through the studies of *Lewis* and *Ferguson* (1950) on the content of plasminogen activator in various organs from the dog,

in which they found a high concentration in the uterus. This observation was later confirmed by *Albrechtsen* (1957 b), who determined the content of tissue activator in the uterus of various species (rat, guinea pig, ox, horse, pig, dog, cat and rabbit) by the aforementioned quantitative and selective method. It was shown that in all the species studied the uterus contained plasminogen activators; high concentrations were observed particularly in the pig and horse. The bovine uterus differed from those of the other species in that it showed only inconsiderable activity, but, on the other hand, it contained trypsin inhibitors of the pulmin type. Moreover, it was shown that the concentration of the tissue activator varies within wide limits, both from species to species and from animal to animal within the same species. In addition, two independent investigations (*Philips, Butler* and *Taylor* 1956, *Albrechtsen* 1957 a) revealed plasminogen activators in the human myometrium. It appears that, although the concentration of the activators shows considerable variations from individual to individual, the myometrium may nevertheless be classified as one of the human tissues which contain the greatest amounts of activator. The concentration in the human myometrium is considerably higher than is seen in animal uteri. The concentration of tissue activator seems to be unrelated to the age of the individual. While, as will be considered later, a hormonal regulation of the endometrial content of tissue activators exists, such a regulation does not seem to occur in the myometrium. This has been confirmed in animal experiments, in which it was shown that the concentration of the tissue activator in the rat uterus does not fall after castration (*Albrechtsen* 1957 c). On the other hand, the total amount of tissue activator in the uterus decreases after castration owing to the atrophy of the organ (table 6).

Fibrinolytic Activity of the Endometrium

The histological structure of the human endometrium and its morphological changes during the menstrual cycle are well known. Studies on the biochemistry of the endometrium are also available; in these, the content of electrolytes, beta glucuronidase, lipids, glycogen, nucleic acids and alkaline phosphatases has been considered (for references, see *Page, Glendening* and *Parkinson* 1951).

It has also been known for a long time that the human endometrium contains components capable of influencing blood clotting.

Thus, saline extracts from the endometrium may shorten the clotting time of normal blood (*Cristea* and *Denk* 1910, *Schickele* 1912, *Bell* 1913, *Whitehouse* 1914 a, b), evidencing that tissue thromboplastin is present. This enzyme is so loosely bound to the tissue that it is present not only in saline extracts, but it may also be active on direct addition of endometrial tissue to human blood (*Whitehouse* 1914 a).

As distinct from the readily accessible thromboplastin, the cells also contain a firmly bound component, which is capable of delaying the clotting of the blood. Extracts of endometrial tissue produced in a Buchner press at a pressure of 400–500 atmospheres may thus delay the clotting of goose plasma (*Schickele* 1912), and similar observations have been made on extracts from the pig uterus (*King* 1921). The anticoagulant agent may be precipitated with acetic acid (*Dienst* 1912) and be destroyed by heating (*Schickele* 1912). The relation between this so-called antithrombin and the later demonstrated fibrinolytic components is unknown.

The knowledge of the fibrin-splitting components in the endometrium dates back to *Halban* and *Frankl* (1910), who demonstrated that endometrial tissue is capable of digesting serum substrates. They assumed that this property was due to a trypsin-like enzyme in the endometrium. This was firmly bound to the cells and could not be extracted by the solvents in common use. These observations were later confirmed (*Caffier* 1930), and fibrin-splitting properties have also been observed in saline extracts (*Huggins, Vail* and *Davis* 1943) and in aqueous extracts from the endometrium (*Page, Glendening* and *Parkinson* 1951). In a series of studies, *Smith* and *Smith* (1940 a, b, 1944, 1945 a, b, c, 1947) concerned themselves with a substance present in menstrual discharge which exerted a pronounced toxic action on rats. This toxin was present in the highest concentration in the cell debris, in which fibrinolytic activity was also observed. A correlation between the toxin and the fibrinolytic components seemed likely, as it might be assumed either that tissue injuries of the endometrium during menstruation gave rise to a liberation of the fibrinolytic components, which then through an action on the endometrial cells excited the formation of the toxin,

or that the toxin and the fibrinolytic components were identical. Fibrin-splitting properties have also been found in endometrial tissue from several species (*Champy* and *Morita* 1928), including the rabbit (*Galstjan* 1933).

As previously pointed out, it is not possible, on the basis of these observations, to infer anything as to the nature of the fibrinolytically active components, since the substrates used for the determination contained both fibrin and plasminogen. However, *Frankl* and *Aschner* (1911) found that aqueous extracts from the human endometrium may split peptone, and it has later been shown that the human endometrium may also split haemoglobin at pH 4.1 (*Stark* and *Vorherr* 1955). These observations suggest that the human endometrium contains proteolytic enzymes (cathepsins).

In addition, more recent investigations have shown that the human endometrium contains a plasminogen activator (*Philips, Butler* and *Taylor* 1956, *Albrechtsen* 1956 a). *Philips* and his coworkers demonstrated that extracts from the endometrium, prepared by the method of *Tagnon* and *Palade* (1950) by grinding and suspension in a 30 % glucose solution, were capable of splitting Armour's fibrin, and that this fibrin-splitting property increased on addition of plasminogen. They therefore concluded that the tissue extracts contained both plasmin and plasminogen activators. However, as the fibrin used contained plasminogen, the direct effect of the tissue extracts on the fibrin cannot be taken as evidence in support of the presence of plasmin, which, as previously mentioned, was emphasised by *Tagnon* and *Palade*. *Albrechtsen*'s studies comprised a total of 67 samples of endometrial tissue obtained by curettage. These were studied by the previously described quantitative and selective method for the determination of the content of tissue activator in fresh tissue (*Astrup* and *Albrechtsen* 1957). The tissue activator was found in all the samples except two (originating from a 56-year-old woman and from a patient with postabortal endometritis). In the determinations of the extracts against heated bovine fibrin (which does not contain plasminogen), no splitting of the fibrin occurred, evidencing that the extracts did not contain fibrinolytic enzymes. The studies of the endometrial tissue activator at various temperatures and pH levels showed agreement with similar studies of the tissue activator in both human and animal

tissues; the activator was relatively stable even at acid pH levels and temperatures of up to 70° C. The endometrial tissue did not contain fibrinolytic inhibitors similar to the trypsin inhibitor of ox-lung tissue (pulmin). However, *Page, Glendening* and *Parkinson* (1951) found that aqueous extracts from the human endometrium may inhibit purified plasmin by a method described by *Guest, Daly, Ware* and *Seegers* (1948). This observation suggests that an antiplasmin is present in the endometrium. In view of the above-mentioned results it must therefore be assumed that this antiplasmin is different from pulmin.

Fibrinolytic Activity of the Endometrium during the Menstrual Cycle

The fibrin-splitting ability of the endometrium varies during the menstrual cycle. As early as 1910, *Halban* and *Frankl* showed that fibrinolysis was at a maximum during the secretory stage and was very slight in the proliferative stage, while activity was absent before menarche and after menopause. These observations were later confirmed by *Caffier* (1930). As previously pointed out, the measuring methods used by these authors (small tissue fragments placed on the serum substrate of Löffler plates with ensuing measurement of the lysed area after incubation) give a possibility of only exploratory experiments. However, subsequent experiments in which better methods were used (*Page, Glendening* and *Parkinson* 1951) confirmed these older observations; but as neither the older nor the more recent methods could be used for qualitative determinations, renewed investigations into these problems were required.

Available qualitative determinations of the concentration of the tissue activator in the human endometrium show good agreement. Thus, *Philips, Butler* and *Taylor* (1956) found that the concentration of tissue activators tended to be higher in the secretory than in the proliferative stage. However, as already mentioned, the method used (*Tagnon* and *Palade* 1950) does not give a reliable quantitative expression of the concentration of the tissue activator, but it has been of value in exploratory experiments. Moreover, the material analysed was very limited, consisting of only three samples from the secretory and four from the proliferative stage. The investigations

performed by *Albrechtsen* (1956 a) during the same period comprised a total of 52 samples of normal or near-normal endometrial tissue. The following results were obtained (figs. 2 and 3):—

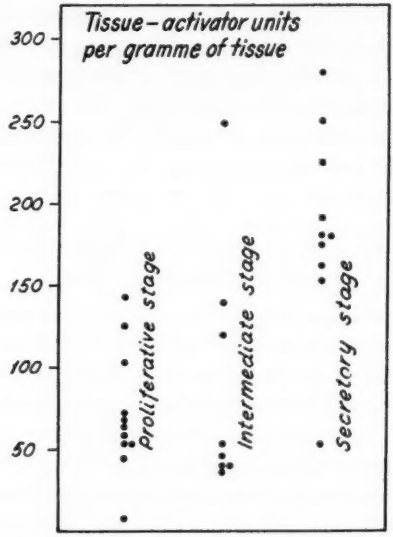

Fig. 2.—Content of tissue activator in normal endometria from women under 40 years of age, expressed in tissue-activator units per gramme of fresh tissue.

In women under 40 years of age, the concentration of the tissue activator in the endometrium was moderate in the proliferative stage (less than 150 units per gramme of tissue), fairly unchanged in the intermediate stage, but considerably higher in the secretory stage. In women over 40 years of age, the concentration was moderate in all three stages. One sample from a woman aged 56 was inactive. Thus, these investigations show that the concentration of the tissue activator in the endometrium increases in the premenstrual stage in young women, but decreases with age.

Certain types of pathological endometria have a high fibrin-splitting capacity. This applies, in particular, to hyperplastic endometria and endometria from women with proliferative bleedings. Thus, *Halban* and *Frankl* (1910) observed that hyperplastic uterine

mucosa lysed serum substrates to a greater extent than normal proliferative mucosa. This observation was later confirmed by *Caffier* (1930) and *Page, Glendening* and *Parkinson* (1951). *Albrechtsen*

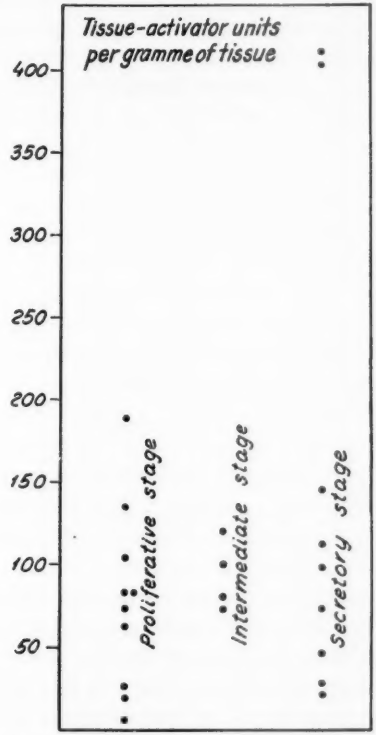

Fig. 3.—Content of tissue activator in normal endometria from women over 40 years of age, expressed in tissue-activator units per gramme of fresh tissue.

(1956 a) showed that this increase in the fibrin-splitting activity of pathological endometria is due to an occasionally considerable increase in the concentration of the tissue activator (table 5).

The fluctuations in the concentration of the tissue activator during the menstrual cycle, the absence of tissue activator in the endometrium after menopause, the decreased concentration in the secretory stage in women over 40 years of age, and the increase in

Table 5.

Content of tissue activator in pathological endometria, expressed in tissue-activator units per gramme of fresh tissue.

Age, in years	Diagnosis	Tissue-activator units per gramme of tissue
43	Proliferative bleeding	432
46	do.	281
34	do.	180
23	do.	180
25	do.	126
37	do.	95
22	Endometrial hyperplasia	213
44	do.	203
49	do.	176
43	do.	113
42	do.	105
24	Chronic endometritis	203
21	Postpartum endometritis	90
56	Endometrium in involution	0
30	Postabortal endometritis	0

the concentration in hyperplastic endometria and in endometria from women with proliferative bleedings are suggestive of a correlation between the fibrinolytic activity and the female sex hormones. Several authors have pointed out that a decreasing oestrogen concentration in the blood might be responsible for an increase in the fibrinolytic activity in the premenstrual stage (*Caffier* 1930, *Smith* and *Smith* 1945 a, c). This hypothesis was later confirmed experimentally by *Page, Glendening* and *Parkinson* (1951), who showed that the concentration of the fibrinolytic agents in the rabbit uterus decreased after castration, remained unchanged after administration of oestrogen or progesterone, but increased some time after the cessation of oestrogen treatment. They concluded that the concentration of "fibrinolytic enzymes" in the rabbit uterus increased at the time when the oestrogen concentration in the circulating blood began to decrease.

This has not been fully confirmed by the present investigations. First, it was found that the fibrinolytic activity in the rabbit uterus, just as in the rat uterus, is not due to a fibrinolytic enzyme, but to a plasminogen activator (*Albrechtsen* 1957 b). Secondly, rat experiments showed that the concentration of plasminogen activator remains unchanged even 4 to 6 weeks after castration (*Albrechtsen* 1957 c). The concentration of the tissue activator also remained unchanged when the rats after castration were treated with oestrogen injections and were killed immediately after the last injection, while they were still in early oestrus. On the other hand, if the animals after the last injection were allowed to survive until late oestrus (checked by the method of Allan Doisy), an increase in the concentration of the tissue activator was observed (table 6).

These last experiments confirm, to some extent, the results obtained by *Page* and his co-workers. In the present experiments, the entire uterus (both myometrium and endometrium) was studied, as it proved technically impossible to separate these two layers and to perform separate analyses. However, the uterine mucosa from a castrated rat is atrophied, for which reason the activity recorded must be due exclusively to the content of activator in the musculature. As no significant difference could be demonstrated between this concentration and that of the uteri of animals in early oestrus, it must be assumed (1) that the content of the activator in the musculature is independent of hormonal influence, and (2) that the content of activator in the oestrual mucosa is very low. This is in agreement with the results obtained in analyses of the human myometrium (*Albrechtsen* 1957 a), which did not reveal any significant difference in the concentration of tissue activator in the myometrium of girls, fertile women and women beyond the reproductive age. Although it was not proved in the investigation considered here, it seems likely that the increase in the concentration of the tissue activator from early to late oestrus is due to an increase in the concentration in the uterine mucosa. If this be so, it would correspond to the increase in the concentration in the human endometrium in the secretory stage. It has not been possible to prove that this assumption is tenable, because methods for a reliable determination of the concentration of tissue activator in so small tissue samples are not available.

Table 6.

Content of tissue activator in the rat uterus at various stages of the oestrous cycle (anoestrus, early oestrus and late oestrus), expressed in tissue-activator units per gramme of tissue and for the entire uterus.

Treatment	Weight of uterus, in mg	Units (and average), per gramme	Units (and average), entire uterus
Untreated controls	427	18 (16)	8 (5)
	325	16	5
	555	6	3
		9	
		18	
		27	
Ovariectomised 4 to 7 weeks (no epithelial cells in vaginal smear). Anoestrus	58	17 (18)	1 (1)
	59	12	1
	55	12	1
	53	12	1
	95	14	1
	60	21	1
	90	23	2
	80	23	2
	100	23	2
	75	10	1
	105	29	3
Ovariectomised; oestradioldipropionate, 5 to 11 injections; killed a few days after last injection. Early oestrus	280	30 (20)	8 (7)
	276	29	8
	310	30	9
	400	30	12
	370	8	3
	380	15	6
	475	10	5
	475	8	4
Ovariectomised; oestradioldipropionate, 2 to 4 injections; killed 17 to 56 days after last injection. Late oestrus	240	43 (53)	10 (15)
	250	56	14
	375	45	17
	280	56	16
	250	96	24
	260	34	9
	320	38	12

Significance of the Tissue Activator during Menstruation

Our knowledge of the histophysiology of menstruation is still incomplete, and the significance of the tissue activator in this process is unknown. It has previously been claimed that the tendency to endometrial oedema and the small bleedings in the tissue during menstruation might be explained by the presence of fibrinolytic ferments, which were assumed to break down the mucosal capillaries (*Halban* and *Frankl* 1910, *Frankl* and *Aschner* 1911, *Caffier* 1930). The menstrual endometrium reveals distinct signs of circulatory necrosis with bleedings in the tissue, oedema, tissue destruction and leukocytic infiltration. This necrosis is presumably to some extent caused by a constriction of the spiral artery (*Markee* 1938). It has been suggested that a correlation exists between this constriction and the decreasing concentration of oestrogenic hormones in the circulating blood in the premenstrual stage, since these hormones are known to be able to produce dilatation of the vessels (*Reynolds* and *Foster* 1940).

Considerations on the significance of the tissue activator in the endometrial changes observed during the shedding of the mucosa can as yet be only of a hypothetical nature. However, it seems to be very likely that the tissue activator exerts an influence, both because it is present in considerable amounts in the mucosa at the time of menstruation, and because it, on account of the cell necrosis, may be released from the cells and exert its action on the surroundings. The demonstration of the tissue activator in menstrual blood shows that such a release occurs (*Albrechtsen* 1956 b). It must be assumed that the tissue activator and the vasoconstriction may be contributory factors in causing bleedings in the tissue in such a manner that the vasoconstriction causes an incipient necrosis with liberation of the tissue activator, and that this then accentuates the tendency to bleedings in the tissue. Both the vasoconstriction and the high concentration of the tissue activator are, perhaps, referable to the decreasing concentration of oestrogens in the circulating blood in the premenstrual stage.

A similar mechanism might be conceived to be at work in other forms of uterine bleedings (proliferative bleedings, endometrial hyperplasia), since the concentration of the tissue activator in the

endometrium is also high in these conditions. This might explain the therapeutic effect of oestrogen injections in certain forms of abnormal uterine bleedings (*Greenblatt* and *Barfield* 1951), since this therapy may control the bleeding partly by a dilative effect on the vessels of the endometrium and thus counteract the circulatory necrosis, and partly by counteracting the increasing concentration of the tissue activator which has been observed in these patients.

Fibrinolytic Activity of the Decidua and Placenta

Opinions are rather divergent as to the fibrinolytic properties of decidual and placental tissues. Early reports stated that trypsin-like enzymes were present in the chorionic epithelium during the first five months of pregnancy (for references, see *Halban* and *Frankl* 1910), and later investigations have shown that the human decidual tissue up to the second month of pregnancy contains fibrin-splitting components (*Caffier* 1930). The fibrin-splitting capacity is reported to persist in the basal layers, whereas it disappears in the superficial layers later in pregnancy. In addition, a plasminogen activator has been revealed in placental tissue from the dog (*Lewis* and *Ferguson* 1950) and the ox (*Albrechtsen* and *Claassen,* unpublished studies), whereas the human placenta is fibrinolytically inactive but contains fibrinolytic inhibitors (*Page, Glendening* and *Parkinson* 1951). Using the method described by *Tagnon* and *Palade* (1950), *Philips, Butler* and *Taylor* (1956) showed that placental tissue (five samples) and decidual tissue (five samples) originating from patients subjected to hysterectomy, as distinct from endometrial and myometrial tissues, did not contain plasminogen activators.

The discordant results and the inadequate technique required that these problems were subjected to renewed investigations. Such an investigation performed by *Albrechtsen* (1956 a) showed that a distinction must be made between normal and pathological decidual tissue and, possibly, between normal and pathological placental tissue. As previous reports do not always state whether the tissues examined were normal or pathological, this alone may give rise to discordant results. *Albrechtsen* performed his studies on decidual tissues from both artificially induced and spontaneous abortions and on normal and pathological placentae. Among 35 samples of decidual

tissue from induced abortions (normal decidua), moderate amounts of plasminogen activator was found in two (i. e. 6 %), while all the other samples were inactive. Thus, plasminogen activator disappears from the endometrium when this is transformed into decidual tissue. However, as all the pregnancies in this series were interrupted after the end of the second month of gestation (mainly for psychiatrical reasons), nothing can be said as to the content of plasminogen activator in early pregnancy. In normal placental tissue, tissue activator was also absent. Samples of decidual tissue from 33 women who had been admitted to hospital with a diagnosis of spontaneous abortion and subjected to operation (evacuation of the uterus) were analysed. Tissue activator was present in 15 cases (i. e. 45 %), sometimes in a very high concentration, while the remaining cases did not reveal any activity. Thus, the content of tissue activator in decidual tissue differed considerably in the group of spontaneous abortions (pathological decidua) and that of induced abortions (normal decidua). In addition, as it must be supposed that a great proportion of the abortions diagnosed as spontaneous are actually artificially induced in some way or other, this difference will be further accentuated, as the latter cases correspond to the induced abortions in the normal series and will be fibrinolytically inactive. It will therefore be reasonable to conclude that the tissue activator occurs in decidual tissue only from actually spontaneous abortions and is, perhaps, as in normal menstruation, of importance for the bleeding in the tissue and hence for the occurrence of abortion. However, the present fairly limited material does not allow of definite conclusions. The interrelationship of the tissue-activator concentration in the endometrium and the occurrence of uterine bleedings on the one hand and this concentration and the hormonal influence on the other may, however, contribute to our understanding of the cause of certain spontaneous abortions of so far unknown aetiology and thus facilitate treatment. If a decreasing oestrogen concentration in the circulating blood, through a circulatory necrosis and liberation of the tissue activator, is capable of inducing uterine bleedings in non-pregnant women, it is possible that a similar mechanism may be at work in pregnant women and thus be the actual cause of certain abortions. Such a theory may very well be compatible with the aforementioned observations and

with the fact that, in women with habitual abortions, continuous oestrogen therapy has often been able to counteract the occurrence of further miscarriages (*Snaith* 1949, and others). Thus, *Snaith* treated a total of 31 patients with threatened abortion with oestrogenic hormones (oestradiol benzoate or stilboestrol, sometimes in combination with some other therapy); this treatment proved effective in 25 cases. According to his report, this result should be considerably better than those obtained by other methods of treatment (progesterone, vitamin E). The causes of the improved results were stated to be: (1) a sedative effect on the uterine musculature and (2) a vasodilative effect resulting in a better blood supply to the foetus and the decidual tissue and in a stimulatory action on the growth of the uterus. The question as to the significance of fibrinolysis in this connexion will require further investigations on the fibrinolytic activity of pathological decidual tissue and determinations of the oestrogen concentration in the blood of patients with habitual abortions. A final clarification of this question must be considered to be of importance in the treatment of these conditions, which are obscure at the present time (*Albrechtsen* 1957 e).

The theory has been advanced that a fibrinolytic process should be instrumental in the separation of the placenta after normal delivery (*Favre-Gilly* 1952). The absence of plasminogen activators in the normal placenta is incompatible with this theory, and further analyses performed on decidual tissue from the placenta and the inside of the uterus failed to lend support to it, since these layers are fibrinolytically inactive (*Albrechtsen* 1957 d).

The aetiology of certain forms of premature placental separation is still unknown. A local fibrinolytic process in the placenta induced by the liberation of tissue activators might have explained this clinical picture, but analyses of a limited number of such placentae have now shown that there are no plasminogen activators in this tissue (*Albrechtsen* 1957 d). However, the aetiology of premature placental separation and its patho-anatomical picture are presumably of a multiform character, for which reason a limited number of negative results are not of decisive importance.

Fibrinolytic Activity of Menstrual Blood

It has not been possible to confirm the observation made by Hippocrates 3000 years ago that clotting of menstrual blood from normal women occurs rapidly, while that from sick women does not clot (*Cowe* 1846). On the other hand, it is generally agreed that normal menstrual blood does not clot either spontaneously or on addition of thrombin. This phenomenon has been studied by many investigators and has given rise to many different hypotheses. It has justly been a matter for surprise that blood from the uterus remains unclotted, while that from almost any other form of bleeding from the normal organism clots on standing. Both haematologists and gynaecologists have concerned themselves with this problem, since its solution would throw further light on the mechanism of coagulation and presumably contribute to the understanding of various pathological uterine bleedings.

In many older textbooks of medicine it is stated that the incoagulability of menstrual blood is due to the alkaline cervical secretion (*Gebhard* 1898). It has proved impossible to trace the origin of this hypothesis, which has incidentally been disproved by a relatively simple experiment in which it was shown that a mixture of cervical mucus and normal fibrinogen-containing blood showed normal clotting (*Birnbaum* and *Osten* 1906).

Others have sought the cause of the incoagulability of menstrual blood in the acid vaginal secretion (*Schroeder* 1913). However, this factor is without importance, since it was later shown experimentally that normal clotting occurs in a mixture of menstrual blood (containing acid vaginal secretion), normal plasma (with fibrinogen) and thrombin (*Bell* 1913).

Several possible explanations of the phenomenon have later been suggested:—

1. Presence of anticoagulant components in menstrual blood.

2. Absence of one or more of the factors which are necessary for coagulation.

3. Presence of active fibrinolytic components in menstrual blood.

These three possibilities are considered in some detail below.

1. Several investigators have advanced the theory that liberation of antithrombin from the endometrium should be responsible for the

incoagulability of menstrual blood (*Schickele* 1911, 1912, *Dienst* 1912, *Zondek* 1921). *Hermstein* (1927) observed that menstrual blood delayed the clotting of normal human blood, evidencing that the former contains an anticoagulant factor, and further showed that this factor could be removed from menstrual plasma by treatment with petroleum ether. Finally, *Greenberg* (1946, 1948) claimed that a relationship existed between the anticoagulant substance he had observed in the myometrium (hysterin) and the incoagulability of menstrual blood.

However, opinions are rather divergent as to whether menstrual blood is capable of delaying the clotting of normal blood. Thus, *Whitehouse* (1914 a, b), *Cristea* and *Denk* (1910) and others observed a reduction in the clotting time in a mixture of menstrual blood and normal human blood, suggesting the presence of thromboplastin.

2. *Birnbaum* and *Osten* (1906) were the first who advanced the theory that inadequate formation of thrombin during menstruation was the cause of the incoagulability of menstrual blood, whereas *Cristea* and *Denk* (1910) assumed that the endometrium was able to retain either thrombin or its precursor. Similar considerations were advanced by *Bell* (1910, 1912). However, no experimental evidence in support of these hypotheses is available. In 1921, *King* claimed that the inability of menstrual blood to clot oxalate plasma was evidence of the absence of thrombin, and advanced the hypothesis that the thrombin by a binding to antithrombin in the uterus is converted into metathrombin. Finally, *Caffier* (1930) postulated that the thrombin should be broken down by the proteolytic components present in the endometrium. All these considerations were of a hypothetical nature.

The finding that fibrinogen is absent in menstrual blood was of greater interest. This observation was first made in haematocolpos blood by *Bell* in 1912 and two years later in normal menstrual blood by *Whitehouse* (1914 a). All subsequent investigators have been able to confirm these observations. On addition of thrombin to menstrual blood no coagulation occurred (*King* 1921, *Lozner, Taylor* and *Taylor* 1942, *Erf* 1952, *Elert* and *Nold* 1956); nor was any precipitates observed after heating of menstrual blood to 60° C. (*King* 1921). However, recalcification of oxalated menstrual blood

resulted in the precipitation of minute amounts of fibrin, evidencing that the plasma contained very little fibrinogen (*Andor* and *Waldbauer* 1928). It has thus been shown experimentally that menstrual blood contains only extremely small amounts of fibrinogen or none at all, and that this is the cause of its inability to clot.

Several theories have been advanced in explanation of this absence of fibrinogen. It has been postulated that the endometrium is capable of retaining the fibrinogen together with the thrombin (*Bell* 1912), but this assumption has been refuted by several investigators. It has thus been shown that through a glass catheter introduced into the uterine cavity during menstruation it is possible to obtain menstrual blood which clots normally, while the blood discharged from the uterus does not clot (*Whitehouse* 1914 a). Moreover, in 120 women with normal menstruation, *Whitehouse* found that the menstrual discharge contained small blood clots in about 50 % of the cases. This was later confirmed by *King* (1921), *Stickel* and *Zondek* (1921), *Andor* and *Waldbauer* (1928), *Lozner, Taylor* and *Taylor* (1942) and *Erf* (1952). This observation suggests that the menstrual blood first clots in the uterine cavity and is then, at least partially, redissolved by some process of a fibrinolytic nature. The blood coming from the endometrium should thus originally possess normal coagulability. It has been assumed that this coagulation in the uterine cavity should be referable to the endometrial content of thromboplastin, which should be liberated during menstruation (*Whitehouse* 1914 a), an assumption which was confirmed by the observation of thromboplastic activity of menstrual blood (*Whitehouse* 1914 a, *Cristea* and *Denk* 1910, *Lozner, Taylor* and *Taylor* 1942, *Huggins, Vail* and *Davis* 1943, *Erf* 1952, *Elert* and *Nold* 1956). The theory of intra-uterine coagulation is further supported by the low prothrombin levels observed in menstrual plasma (*Lozner, Taylor* and *Taylor* 1942, *Elert* and *Nold* 1956), although these low values may obviously be referable to other factors (for example, fibrinolytic activity).

3. The normal clotting of menstrual blood obtained direct from the uterine cavity and the absence of fibrinogen in the menstrual discharge have obviously directed attention to the possibility of a fibrinolytic process as the cause of the redissolution of the fibrin. Thus, *Whitehouse* (1914 a) was the first to show that a mixture of

menstrual blood and normal blood after clotting liquefied again. This was taken as evidence of the presence of active fibrinolytic components in the menstrual blood—an interpretation which was later confirmed both as far as menstrual blood (*Smith* and *Smith* 1945 a, *Elert* and *Nold* 1956) and haematocolpos blood (*Kross* 1924) are concerned. Further evidence in support of this theory has been revealed by the observation of increased proteolysis of menstrual blood (*Andor* and *Waldbauer* 1928). The proteolytic components of menstrual blood are unable to attack the fibrinogen in oxalate plasma (*Huggins, Vail* and *Davis* 1943) and are found in the globulin fraction (*Smith* and *Smith* 1945 a). Certain observations suggest that the fibrinolytic components and the toxin found in menstrual discharge by *Smith* and *Smith* are identical (*O. W. Smith* 1947). However, *Lozner, Taylor* and *Taylor* (1942) could not invariably demonstrate fibrinolytic activity in menstrual blood, but as the information as to their experimental technique is rather incomplete, their results cannot be definitely assessed.

Animal experiments have confirmed the results obtained in analyses of human menstrual blood. Thus, secretions from the rat uterus can redissolve fibrin clots of rat, guinea-pig and human blood, in certain cases in the course of only 15 minutes (*Kross* 1923, *Huggins, Vail* and *Davis* 1943).

On the basis of the above-mentioned observations it has been concluded that menstrual blood contains a fibrinolytic enzyme. However, this assumption was not conclusively proved, since the fibrinolytic activity in all the experiments was measured against plasminogen-containing substrates. It was therefore necessary to subject this problem to renewed investigations. *Albrechtsen* (1956 b) performed his experiments on nine women with normal menstruation; menstrual blood was collected in an occlusive pessary inserted into the vagina for from 4 to 8 hours. The blood was then centrifuged and the supernatant precipitated iso-electrically at low ionic strength and pH 5.4 (*Milstone* 1941) in order to remove the inhibitors of the albumin fraction. After redissolution of the precipitate in a buffer solution the activity was determined against both heated and unheated bovine fibrin by the fibrin-plate method (*Astrup* and *Müllertz* 1952, *Lassen* 1952). All the samples showed fibrinolytic activity, and further analysis of the components entering into the

fibrinolytic process revealed the presence of a fibrinolytic enzyme (demonstrated by digestion of the substrate of the heated plates, in which there is no plasminogen) and large amounts of plasminogen activators (demonstrated by a considerably more potent digestion of the plasminogen-containing substrate of the ordinary fibrin plates and by an increase in the activity against heated fibrin on addition of purified plasminogen, which is inactive in itself). Further addition of streptokinase to the menstrual blood resulted in an increase in the activity against untreated fibrin, while no increase in the activity against heated fibrin occurred in this mixture. This shows that menstrual blood contains a precursor of a plasminogen activator which may be transformed into the plasminogen activator itself by means of streptokinase (*Müllertz* and *Lassen* 1953), and that there is no plasminogen in menstrual blood, since this, if it had been present, would have been converted into plasmin owing to the presence of large amounts of plasminogen activators, and thus would have caused an increase in the activity against heated fibrin. It was also shown that the fibrinolytic activity of menstrual blood decreases on the second day of menstruation, which confirms the old observation that the proteolysis of menstrual blood decreases after the first day of menstruation (*Andor* and *Waldbauer* 1928). On the basis of these observations it was concluded that the fibrinolytic activity of menstrual blood is due to a plasminogen activator which has transformed all the plasminogen of the menstrual blood into plasmin.

The underlying mechanism of the fibrinolytic activity has been the subject of several investigations. Two views, which are considered in some detail below, have been advanced, viz.

1. The fibrinolysis of menstrual blood is a manifestation of a fibrinolytic process of a general nature also comprising the circulating blood.

2. The fibrinolysis of menstrual blood is a local phenomenon confined to the uterine cavity.

1. A number of investigators have studied the coagulability of circulating blood during menstruation. The purpose of these studies was to prove or disprove the postulation that women during menstruation have a generalised bleeding tendency, for which reason surgical intervention should be avoided during the menstrual periods. The results obtained have often been contradictory, and this applies

also to the studies performed with a view to fibrinolytic activity. While increased proteolysis of menstrual blood has been demonstrated, it has not been possible to disclose increased proteolytic activity in the circulating blood during menstruation (*Andor* and *Waldbauer* 1928). *Schittenhelm* and *Lutter* (1906), *Macfarlane* and *Biggs* (1946) and *Elghammer, Burton, Grossmann, Koff, Moulder* and *Garrot* (1949) failed to demonstrate increased fibrinolytic activity of the circulating blood during menstruation, and our own investigations have also been negative. It has thus appeared that iso-electrically precipitated plasma from menstruating women is unable to lyse bovine fibrin (fibrin-plate method) more vigorously than is seen in corresponding samples of plasma from non-menstruating women. However, increased fibrinolytic activity of the blood during menstruation has been reported by other investigators. Thus, *Smith* and *Smith* (1945 a) observed that a mixture of human oxalate plasma and serum from the blood of menstruating women after clotting was redissolved, and this observation was later confirmed by *Willson* and *Munnell* (1946), *Marx* and *Rovatti* (1952) and, more recently, by *Dausset, Bergerot-Blondel* and *Colin* (1957), who in 16 samples out of 41 found complete or partial fibrinolysis in a mixture of peripheral blood, physiological saline and human inactive serum when the peripheral blood was withdrawn during menstruation. On the other hand, three samples showed fibrinolytic activity on the 14th or 15th day of the menstrual cycle, whereas the remaining samples did not reveal any fibrinolytic activity.

At the present time it is difficult to explain these discrepancies. The methods available for the determination of fibrinolytic activity in blood are inadequate. In particular, great difficulties have been encountered in separating the fibrinolytically active components from the fibrinolytic inhibitors of the blood, for which reason quantitative determinations have been impossible. A final solution of the problem cannot be expected until it becomes possible to isolate the active and inhibitory components and determine them separately. However, judging from available studies it cannot be doubted that the fibrinolytic activity of the circulating blood during menstruation is not a constant phenomenon, and that it is far less than the activity of menstrual blood, for which reason the latter

activity cannot be taken as an expression of a general increase in the fibrinolytic activity of the blood.

2. Early experience had shown a difference between menstrual blood and circulating blood during menstruation. Thus, *Cristea* and *Denk* (1910) found that blood secured from small incisions in the cervix uteri during menstruation clotted normally, for which reason they concluded that the coagulation defect of menstrual blood was referable to components from the endometrium. Similar observations were made by *Stickel* and *Zondek* (1921) and *Zondek* (1921). It was therefore reasonable to assume that there was a relationship between the fibrinolytic activity of the endometrium and that of menstrual blood. Although conclusive proof of the actual existence of such a relationship has been furnished only be recent investigations (*Albrechtsen* 1956 b), several investigators had previously considered this possibility. Thus, *Halban* and *Frankl* (1910) assumed that there was a relationship between the trypsin-like enzyme they found in the endometrium and the incoagulability of menstrual blood, because they showed that addition of trypsin to ordinary blood completely abolished its ability to clot. Similar considerations were later advanced by *Whitehouse* (1914 a, b), *Kross* (1923), *Andor* and *Waldbauer* (1928), *Caffier* (1930), *Smith* and *Smith* (1945 a) and *Page; Glendening* and *Parkinson* (1951). However, the lack of knowledge of the factors which are involved in the fibrinolytic activity of both the endometrium and menstrual blood makes it impossible to identify these two processes.

Now we know that the fibrinolytic activity of the endometrium is due to a plasminogen activator of the same stability as the tissue activator in other human tissues, and that menstrual blood also contains a plasminogen activator. According to our present knowledge of the components of the system of fibrinolytic enzymes, two different plasminogen activators are present in the organism, viz. the tissue activator of plasminogen and the labile plasminogen activator in the blood. *Albrechtsen* (1956 b) showed that the plasminogen activator of menstrual blood is of the same type as that of the endometrium, as it is relatively stable, especially at acid reaction, and thus differs from the labile plasminogen activator in the blood. This observation in combination with the aforementioned results as

to the plasminogen activator of the endometrium (in particular, the favourable conditions for its liberation which are present during menstruation) strongly suggests that the plasminogen activator of the endometrium is liberated during the menstrual period and is the cause of the fibrinolytic activity of menstrual blood. The decrease in the fibrinolytic activity of menstrual blood observed on the second day of menstruation lends further support to the assumption that the activity originates from the endometrium, since the greater part of the endometrium is shed from the uterine cavity at that time (*Linde* and *Novak* 1924). Thus, it is now possible to explain the old observation made by *Andor* and *Waldbauer* in 1928, viz. that the proteolytic activity of menstrual blood seems to depend on the amount of endometrial tissue, and that the presence of small amounts of endometrial tissue is often combined with the presence of clots in the menstrual blood evidencing diminished proteolytic activity. It is now also explicable that the blood discharged from the uterus in relation to normal delivery clots normally (*Greenberg* 1945), since the bleeding does not come from a tissue-activator-containing endometrium, but from decidual and placental tissues, which are now known to be fibrinolytically inactive.

It may thus be concluded that two biological mechanisms are at work in the course of normal menstruation, both excited by the liberation of tissue components from the damaged endometrium. The thromboplastic activity causes coagulation of the blood in the uterine cavity and may thus be assumed to be of significance in the limitation of the bleeding (*Whitehouse* 1914 a). The fibrinolytic activity causes a redissolution of such clots and must therefore be assumed to constitute the mechanism by which the organism relieves itself of the menstrual discharge. The local conditions in the uterus during menstruation may correspond to those occurring in other parts of the organism, when, for example, local tissue injuries give rise to liberation of tissue components with both thromboplastic and fibrinolytic properties. Thus, analyses of the local fibrinolytic conditions in the uterus give a possibility of a better understanding of the fibrinolytic processes which under certain circumstances (tissue injuries, etc.) occur in other parts of the organism.

An increase in the volume of blood, which is seen in certain pathological conditions (profuse metrorrhagia and menorrhagia),

causes a disturbance in the interaction between coagulation and fibrinolysis in favour of increased coagulation. This results in the formation of clots in the menstrual blood. It may be conceived that the amount of plasminogen activator available in the endometrium is insufficient to bring about a redissolution of the large amounts of fibrin which are formed in such conditions, even though the endometrium in these cases has proved to contain very large quantities of the tissue activator. It is also possible that the quantity of plasmin formed is insufficient because of too small amounts of plasminogen in the fibrin. In very profuse bleedings the menstrual blood may occasionally contain so large clots that their passage through the canal of the cervix seems unlikely. Such clots must therefore have been formed in the vagina. In such cases the bleeding must have been so intense that neither the coagulation process nor the fibrinolytic process has had any possibility of occurring within the uterus. According to these considerations, a history of the presence of large "lumps" in the blood (in non-pregnant patients) must be of importance in the evaluation of the intensity of the bleeding.

However, at the present time it is not unanimously agreed that menstrual blood first clots in the uterine cavity and then undergoes fibrinolysis. Thus, *Beller* and *Graf* (1957) have recently advanced the theory that the plasmin of menstrual blood directly attacks the fibrinogen (fibrinogenolysis), and hence that no preceding coagulation occurs. This theory is mainly based upon the differences which they observed in the content of coagulant components in menstrual blood and normal blood serum; these differences are claimed to be incompatible with a preceding process of coagulation. As, however, the process of coagulation and that of fibrinolysis start at the same time, it is obviously difficult to determine what actually happens. Another factor which is also of decisive importance in the solution of the problem fibrinolysis versus fibrinogenolysis is the presence of fibrinolytic inhibitors in menstrual blood, since these will to a certain extent counteract fibrinogenolysis, whereas their influence on fibrinolysis will be of only secondary importance because of the binding of the plasmin to the fibrin (see *Müllertz* 1956).

The incoagulability of menstrual blood is thus due to an afibrinogenaemia (or hypofibrinogenaemia) caused by the combined effect of thromboplastic and fibrinolytic components which have been liberated from the endometrium. In addition, clinical studies during recent years have called attention to a hypo- or afibrinogenaemia of a more generalised nature in the circulating blood frequently occurring in certain obstetrical complications. The cause of these conditions is as yet only partially known, but it seems possible to draw certain parallels between local and generalised afibrinogenaemia.

It is outside the scope of this monograph to review the voluminous literature which is available as to hypo- and afibrinogenaemia in relation to obstetrical complications. During recent years, this subject has been surveyed by a large number of authors (*Favre-Gilly, Potton* and *Potton-Lafuma* 1952, *Jürgens* and *Stein* 1954, *Stefanini* and *Dameshek* 1955, *Ratnoff, Pritchard* and *Colopy* 1955, *Astrup* and *Albrechtsen* 1956, *Beller* 1957, and others). As it has, however, been claimed that the presence of fibrinolytic components in the uterus might be conceived to be of importance in this clinical picture, it will here be reasonable to recapitulate the problem, especially as far as the trigger mechanism is concerned.

Hypo- or afibrinogenaemia is most frequently observed in relation to premature separation of the placenta (in particular, in uteroplacental apoplexy), but also in amniotic-fluid infusion (see later), intra-uterine foetal death (of more than 6 to 8 weeks' duration), placenta accreta, toxaemia of pregnancy, eclampsia, Caesarian section, abortions, hydatid moles, extra-uterine pregnancies, forceps deliveries and sometimes in relation to otherwise perfectly normal deliveries.

Clinically, the conditions are usually characterised by their acute onset, but occasionally, especially in relation to intra-uterine foetal death, a more protracted course may be seen. The pathological condition is often preceded by a shocklike state with signs of cardiac failure. This is followed by haemorrhagic diathesis with discharge of incoagulable blood from the uterus or, in severe cases, by a generalised tendency to bleedings from the skin and mucosae. Without adequate treatment, the condition will usually run a fatal course,

either because of the primary shock or because of a subsequent haemorrhagic shock. Analyses of blood from these patients have in most cases shown that fibrinogen is absent or present in only very considerably reduced amounts (from 1 to 100 mg%), but in exceptional cases a normal fibrinogen concentration has yet been demonstrated.

In the explanation of this clinical picture various possibilities must be considered. Theoretically, the lack of fibrinogen in the blood may be conceived to be due to one or more of the following four processes, which are considered in some detail below:—

1. Intravascular transformation of the fibrinogen into fibrin.
2. Breakdown of the fibrinogen of the blood in the vascular bed (fibrinogenolysis).
3. A combination of intravascular fibrin formation and fibrinolysis.
4. Diminished formation of fibrinogen in the organism due to liver injury.

1. Intravascular transformation of the fibrinogen of the blood into fibrin has been conceived to occur by infusion of thromboplastin from the decidua and placenta. A number of experimental and clinical data are in favour of this theory. It has been shown that the placenta and decidua are the tissues of the human organism which contain the highest concentrations of thromboplastin (for references, see *Beller* 1957), and that injection of either thromboplastin or placental extracts into the vascular bed results in massive pulmonary embolism with immediate death of the experimental animal or in a reduction of the fibrinogen concentration of the blood with haemorrhagic diathesis, irrespective of the amount injected (*Jürgens* and *Studer* 1948, *Schneider* 1954 a). In addition, pulmonary fibrin emboli have occasionally been demonstrated in patients in whom acute afibrinogenaemia has arisen during labour. On the basis of these observations, *Schneider* (1954 b) advanced the hypothesis that the tissue thromboplastin of the placenta and decidua in these pathological conditions enters the maternal blood stream and gives rise to a transformation of the fibrinogen into fibrin intravascularly. This theory is capable of explaining both the diminished fibrinogen content of the blood and the initial shock state (so-called obstetrical shock). The prerequisites for such a thromboplastin infusion should

be present if the maternal blood under pressure is pumped into a retroplacental haematoma, since this may cause a rupture of the decidua, so that tissue extract may enter the maternal circulation (hydraulic pump). The theory is thus supported by both experimental and clinical evidence. However, the reason why it has not been generally accepted must be sought in the fact that such intravascular fibrin emboli have been demonstrated at autopsy only in a fairly limited number of cases.

2. The observation of strong fibrinolytic activity in the blood in relation to the above-mentioned obstetrical complications (*Willson* and *Munnell* 1946, *Soulier, Petit* and *Bolloch* 1952, *Favre-Gilly* 1952, *Lhoiry* and *Fayet* 1954, *Scott, Matthews, Butterworth* and *Frommeyer* 1954, *Lavelle* 1955, and others) has led to the theory that fibrinolytically active substances through a digestion of both fibrinogen and fibrin should be the cause of haemorrhagic diathesis. As haemorrhagic diathesis has occasionally been observed in obstetrical patients with a normal blood concentration of fibrinogen, but with moderate fibrinolytic activity (*Scott* et al.), a thromboplastin infusion cannot explain the clinical picture, whereas fibrinolytic activity may be the underlying cause by redissolving the fibrin deposits formed for the purpose of haemostasis. However, it must be emphasised that in several cases it has not been possible to demonstrate fibrinolytic activity in the blood of these patients, but as the methods available for the determination of fibrinolytic activity in the blood are very inadequate, such negative findings must be evaluated with some reserve. The underlying mechanism of the activation of fibrinolysis in these conditions is unknown. It has been claimed that liberation of fibrinolytically active components from the human myometrium should be of importance in the process (*Moloney, Egan* and *Gorman* 1949, *Mathey, Daumet, Soulier, Bolloch* and *Fayet* 1950, *Ambre, Chattot* and *Gerbay* 1952, *Soulier, Petit* and *Bolloch* 1952, *Favre-Gilly* 1952, *Guilhem, Pontonnier* and *Boisson* 1952, *Soulier* 1953, *Philips, Butler* and *Taylor* 1956, *Astrup* and *Albrechtsen* 1956, and others), but evidence in support of this theory is not available. However, in several of the aforementioned complications the conditions for a liberation of the tissue activator (tissue injuries, cell necrosis) are present, and this in combination with a relatively high tissue-activator concentration in the myo-

metrium is in support of the above-mentioned theory. On the other hand, there is no possibility of an activation of fibrinolysis from the placenta or decidua, which are both fibrinolytically inactive.

3. Thus, it appears that each of the two above-mentioned theories may explain some, but not all, of the phenomena observed in clinical practice. It has therefore now been conceived that both processes should be involved. It has been thought that the thromboplastin infusion with subsequent intravascular coagulation should be the primary factor, and that this in an as yet unknown manner should be the cause of an activation of fibrinolysis. However, it does not seem unreasonable to assume that both thromboplastic and fibrinolytic components may be released from the injured tissues at the same time, and both exert their action on the circulating blood, so that the afibrinogenaemia of the circulating blood may be conceived to have arisen by a mechanism similar to that of afibrinogenaemia of menstrual blood.

4. The theory as to liver injury with diminished formation of fibrinogen seems less likely. A few cases may be referable to injury of the hepatic parenchyma (*Burger* 1950, *Lockard, Ratnoff* and *Hartmann* 1950), but in the vast majority of cases on record the liver function was normal. In addition, it is known that in patients who were successfully tided over the acute phase, the blood-fibrinogen level rapidly returned to normal, for which reason it must be assumed that normal liver function is present.

While it has thus been generally accepted, first, that the blood-fibrinogen level is lowered in most cases, and, secondly, that evidence of fibrinolytic activity is often present, it is as yet uncertain whether or not other coagulation factors may be changed. In a few studies (*Roemer* and *Beller* 1955, and others), a reduction in the concentrations of prothrombin, factor V and factor VII was observed. It is unknown whether this is due to a splitting-up of these components referable to fibrinolysis (*Seegers* and *Loomis* 1946) or to increased formation of thrombin during the vigorous coagulation.

Thus, it is of both practical and theoretical importance to clarify the problems outlined here. Effective treatment of haemorrhagic diathesis presupposes a detailed knowledge of the trigger mechanism. Experience has certainly shown that injection of large amounts of fibrinogen in combination with blood transfusions may counteract

the bleeding tendency (for references, see *Weber* and *Paxson* 1954, *Albrechtsen, Storm* and *Trolle* 1955 c), but a beneficial effect of this treatment can be obtained only when the fibrinogen administered is not transformed into fibrin with subsequent development of fresh fibrin emboli, or is split up by fibrinolytically active substances. It must thus be possible to eliminate both the source of thromboplastin and fibrinolysis. If, as assumed, the thromboplastin is released from the placenta and decidua, prompt evacuation of the uterus will be sufficient to prevent a new infusion into the maternal circulation. It is attended with greater difficulties to combat fibrinolysis. Here two possibilities are open:—

1. The fibrinolytic activity may be counteracted by injection of fibrinolytic inhibitors, such as serum albumins (*Scott, Matthews, Butterworth* and *Frommeyer* 1954).

2. The cause of fibrinolysis may be removed. As it is not as yet known with certainty whether the tissue activator is responsible for the increased fibrinolysis, it is an open question whether or not hysterectomy should be recommended, especially because the operation must then be performed on profusely bleeding patients. Blood transfusions and fibrinogen injections during operation may obviously lessen the risk involved in such surgical measures.

Chapter III

FIBRINOLYTIC ACTIVITY OF THE PROSTATE AND SEMINAL FLUID

Fibrinolytic Activity of the Prostate

Only relatively recently has attention been focused on the fibrinolytic conditions of the prostate. It was first shown that saline extracts from the human prostate dissolved human fibrin more rapidly than similar extracts from the human endometrium, evidencing that a protease was present (*Huggins, Vail* and *Davis* 1943). Similar observations were made by other investigators (*Kaulla* and *Shettles* 1953, *Ying, Day, Whitmore* and *Tagnon* 1956). However, on the basis of such experiments it is not possible to determine the nature of the fibrinolytic components present in the prostate, since the human fibrin used is now known to contain plasminogen, for which reason the measured activity may be due to both proteolytic enzymes and to plasminogen activators. The studies which have been performed in order to determine the character of the fibrinolytic agents of the prostate show distinct discrepancies. *Karhausen* and *Tagnon* (1956) thus found that at 37° C. and pH 7.2–7.4, phosphate-buffer extracts from human hypertrophic prostates break down casein under the formation of tyrosine. On addition of human plasminogen to the prostatic extract, its casein-splitting power was not increased, whereas it was increased when streptokinase was also added. According to our present conception of streptokinase as an activator for the plasminogen pro-activator of the blood (*Müllertz* and *Lassen* 1953), the last of these observations may be explained either by the presence of such pro-activators in the plasminogen or by the presence of blood in the phosphate-buffer extracts, but the above-mentioned authors interpreted the observation as a direct effect of streptokinase

on the plasminogen added. These studies thus show that a protease is present, and that plasminogen activators are absent in the phosphate-buffer extracts. *Albrechtsen* (1957 a) was unable to confirm these observations. Using the quantitative and selective method for the determination of the content of plasminogen activators in fresh tissue described by *Astrup* and *Albrechtsen* (1957), a high concentration of such tissue activators was demonstrated both in the human prostate (*Albrechtsen* 1957 a) and in prostatic tissue from a number of animal species (horse, dog, cat, ox and pig) (*Albrechtsen* 1957 b). By acid precipitation at pH 1.0 of a potassium-thiocyanate extract from prostatic tissue from human cadavers and from the above-mentioned animals (removed immediately after the animals had been killed), a solution was prepared which was able to digest bovine fibrin (fibrin-plate method), but not heated bovine fibrin, which does not contain plasminogen. The concentration observed in the human prostate shows that this gland is one of the organs in the human organism which have the highest content of the tissue activator. These results are widely different from those obtained by *Karhausen* and *Tagnon,* and the discrepancy is at present unexplained. Supplementary studies on these conditions (*Rasmussen, Albrechtsen* and *Astrup* 1957, *Rasmussen* and *Albrechtsen* 1958) confirmed that the fibrinolytic activity of the prostate is due to a plasminogen activator and not to a protease. It was shown that it is possible, both by physiological saline and by 2-M potassium thiocyanate, to extract components which are capable of digesting plasminogen-containing bovine fibrin, but not plasminogen-free bovine fibrin. Studies on the stability of this plasminogen activator at various temperatures and pH levels further suggested that both a plasminogen activator of the stable tissue-activator type and another of the more labile blood type are present in saline extracts, while subsequent extraction with potassium thiocyanate of the tissue which had previously been extracted with saline results in a solution containing only the plasminogen activator of the stable type. However, these results must be supplemented by further experiments, since it cannot be excluded that the presence of other components in the saline extracts (e. g., pepsin) may exert an influence on the stability observed.

Fibrinolytic activity has been observed not only in normal and

hypertrophied prostatic tissue, but also in cancer tissue from the prostate and in metastases from such tumours (*Tagnon, Schulman, Whitmore* and *Leone* 1953, *Tagnon, Whitmore, Schulman* and *Kravitz* 1953, *Tagnon* 1954, 1955). In their experiments, the tumour tissue was homogenised, extracted with phosphate buffer and physiological saline at pH 7.4; the ability of this extract to digest bovine fibrin treated with radio-active iodine by a method devised by *Shulman* and *Tagnon* (1950) was then measured. This fibrin-splitting power was taken as evidence of the presence of proteolytic enzymes in the tissue; but this conclusion does not seem to be correct owing to the content of plasminogen in the substrates used. These extracts could also lyse human fibrin and prolong the prothrombin time in freshly prepared human citrated plasma.

Fibrinolytic Activity of Prostatic Secretions and of Seminal Fluid

Seminal fluid can dissolve human fibrin (*Huggins* and *Neal* 1942). This fibrinolytic activity is localised in the plasma, but is absent in the spermatozoa, since it is preserved after passing the fluid through a Seitz filter. The activity is lost after heating to 70° C. for 5 minutes, but is not affected by temperatures of about 60° C. Prostatic secretions are also fibrinolytically active. Later studies have revealed a fundamental difference between seminal fluids from man and the dog; the former is characterised by a high content of fibrin-splitting components, while the latter chiefly contains components with fibrinogen-splitting properties (fibrinogenases) (*Huggins* and *Vail* 1943, *Huggins* 1945). In the experiments performed by these authors the fibrinolytic activity was measured against fibrin substrates, which are now known to contain plasminogen, for which reason they do not give any possibility of a closer analysis of the active components. The fibrinolytically active components of seminal fluid have later been analysed in greater detail (*Kaulla* and *Shettles* 1953, 1954). These analyses showed that a mixture of human semen and bovine fibrinogen (Armour) after clotting with thrombin lysed again, and that this lysis increased after addition of streptokinase. Addition of human plasminogen to the semen resulted in increasing fibrinolytic activity against bovine fibrin, with maximum activity

after the lapse of from 4 to 5 hours. Increasing amounts of seminal fluid in these mixtures inhibited the fibrinolytic process. From these studies it was concluded that human seminal fluid contains (1) plasmin, (2) plasminogen activators, (3) fibrinolytic inhibitors, and (4) a factor which can be activated by streptokinase. More recent investigations by *Lundquist, Thorsteinsson* and *Buus* (1955) confirmed the presence of plasminogen activators in seminal fluid by the fibrin-plate method; they demonstrated that plasminogen-containing bovine fibrin was digested, whereas no digestion of heated, plasminogen-free fibrin occurred.

Experiments by *Rasmussen, Albrechtsen* and *Astrup* (1957) and *Rasmussen* and *Albrechtsen* (1958) showed that human seminal fluid reacting with heat-treated bovine fibrin (fibrin-plate method) exhibits variable, but usually slight, activity, indicating a low content of a fibrinolytic enzyme. Addition of plasminogen, which is inactive by itself, resulted in a considerable increase in this activity on the heat-treated plates; the samples were also highly active against untreated bovine fibrin, which contains plasminogen. These experiments show that a plasminogen activator is present in seminal fluid. Addition of streptokinase gave variable results. In some cases a slight increase in the activity occurred, while the activity remained unchanged in others. According to our present conception of streptokinase as an activator of the plasminogen pro-activator (*Müllertz* and *Lassen* 1953), these experiments show that such pro-activators are usually absent, but occasionally present in very small amounts, in seminal fluid. Moreover, a high content of trypsin inhibitors, localised to the albumin fraction, was revealed. These inhibitors could be separated from the fibrinolytically active components by iso-electric precipitation with acetic acid at pH 5.3 and low ionic strength (*Milstone* 1941).

It is reasonable to assume that a correlation exists between the fibrinolytic activities of prostatic tissue and seminal fluid. Several investigators have thus claimed that the fibrinolytic components of seminal fluid must have been released from the prostatic tissue in the form of a secretory product (*Huggins* and *Neal* 1942, *Lundquist* 1953, *Kaulla* and *Shettles* 1953, *Karhausen* and *Tagnon* 1955). *Huggins* and *McDonald* (1944) demonstrated a direct relationship between the number of leukocytes in the prostatic secretion from

patients with chronic prostatitis and the fibrinolytic activity, and therefore concluded that a quantitative determination of the fibrinolytic activity of seminal fluid could be used to assess the secretion from the prostate. *Ying, Day, Whitmore* and *Tagnon* (1956) likewise found fibrinolytic activity in prostatic secretions (collected after massage of the prostate), but semen specimens from the same person showed in some cases greater activity than the prostatic secretions. In an attempt to make this finding harmonise with the above-mentioned theory it has been assumed that so-called "accelerators" in the seminal fluid should be able to increase fibrinolysis, but this assumption has not been proved experimentally. It has also been studied if there should be a correlation between the fibrinolytic activity of the seminal fluid and the size of the prostate (estimated by rectal examination), but owing to wide individual variations in the fibrinolytic activity such a correlation could not be demonstrated. Finally, *Harvey* (1949) concluded that the fibrinolytic activity of the seminal fluid was independent of the volume of the prostatic secretion, or that the fibrinolytic components were present only in a small fraction of the prostatic secretion, because he was unable to demonstrate any definite interrelationship between the fibrinolytic activity and the volume of the seminal fluid. Thus it has not been conclusively proved that the fibrinolytic activity of the seminal fluid originates exclusively from the prostate.

Rasmussen, Albrechtsen and *Astrup* (1957) and *Rasmussen* and *Albrechtsen* (1958) approached the problem from a different angle. Two possibilities are open, viz. that the plasminogen activator of the seminal fluid originates either from the blood (and is thus labile, especially at acid reaction) or from the tissue (and is thus stable at acid reaction). By analyses of the plasminogen activator of seminal fluid at different temperatures and different pH levels it should be feasible to distinguish between these two possibilities. Our investigations have now shown that the plasminogen activator of seminal fluid is relatively stable within the pH range from 5 to 9 and is thus similar to the tissue activator, while it is rapidly destroyed at pH levels from 4 downwards, and at these reactions show agreement with the labile plasminogen activator of the blood. However, studies by *Lundquist* and *Seedorff* (1952) showed that human semen contains pepsinogen, which at acid reaction is converted into pepsin. The

presence of this protease in seminal fluid might thus explain the lability of the plasminogen activators at this reaction. Moreover, *Alkjær* and *Astrup* (1957) demonstrated that the stability of the tissue activator from pig hearts at acid reaction is changed into a pronounced lability by addition of pepsin. In view of this finding we made an attempt to isolate the plasminogen activator from seminal fluid by absorption on silica gel and subsequent eluation with ammonia. The plasminogen activator in this solution was distinctly more stable than that in the seminal fluid itself, especially at acid reaction. This suggests that the plasminogen activator in seminal fluid is of the same type as the tissue activator, and must thus be presumed to originate from the prostatic tissue. However, the isolation of the plasminogen activator by the above-mentioned method is not quantitative, for which reason it is impossible to decide whether seminal fluid also contains a labile plasminogen activator similar to that of the blood.

Significance of the Fibrinolytic Activity of the Prostate and Seminal Fluid

The significance of the plasminogen activator present in the prostate and seminal fluid is as yet unknown. It has been claimed that some connexion should exist between fibrinolysis and fertilisation, partly because the fibrinolytic activity keeps the seminal fluid in a liquid state, and partly because this activity may influence the power of the spermatozoa to penetrate the zona pellucida (*Kaulla* and *Shettles* 1954). However, these considerations are merely of a hypothetical nature and have not been supported by experimental evidence.

Other investigators have advanced theories as to the so-called "internal secretion" of the prostate; it has been thought that the fibrinolytically active components of the gland in certain pathological conditions should be liberated from the tissue and enter the circulating blood. Thus, it has been observed (*Tagnon, Whitmore* and *Shulman* 1952, *Tagnon, Whitmore, Schulman* and *Kravitz* 1953, *Tagnon, Schulman, Whitmore* and *Leone* 1953, *Tagnon* 1954, 1955) that certain forms of metastasising cancer of the prostate are complicated by haemorrhagic manifestations (bleedings from the

skin and mucosae) which are due to fibrinolytic activity in the blood. This fibrinolysis was estimated by the power of the plasma to dissolve fibrin containing radio-active iodine and expressed by the amount of liberated iodine after incubation (*Shulman* and *Tagnon* 1950). The fibrinolytic activity was accompanied by diminished fibrinogen values and prolonged prothrombin time. These values returned to normal after treatment with oestrogenic hormones, while testosterone had the opposite effect. These observations combined with the finding that the tissue extracts (both from carcinomata of the prostate and their metastases) were able to reduce the fibrinogen content and prolong the prothrombin time of normal blood in vitro led to the theory that the fibrinolytic components present in the cancer tissue should be the cause of the fibrinolysis observed in the blood of these patients. Similar observations have later been made by other investigators (*Crane, Ware* and *Hamilton* 1955, and others), and fibrinolytic activity in the blood, diminished fibrinogen levels and haemorrhagic manifestations have been encountered after prostatectomy in patients with benign hypertrophy of the gland (*Scott, Matthews, Butterworth* and *Frommeyer* 1954). Both in these operations and in cancer of the prostate, the prerequisites for a release of the tissue activator (destruction of tissue or cells) are present. It may also be assumed that the profuse local bleedings which are often encountered after prostatectomy are due to a mechanism of this nature.

Some parallelism seems to exist between the hormonal influence on fibrinolysis in the prostate and the uterus. Thus, a decreasing oestrogen concentration results in increasing fibrinolytic activity in the circulating blood in prostatic patients, while a similar decrease in the oestrogen concentration gives rise to increased fibrinolytic activity in the endometrium and menstrual blood in females. These observations are in harmony with the fact that *Tagnon, Ying, Whitmore* and *Day* (1956) found decreasing fibrinolytic activity in the prostatic secretion in patients under oestrogen treatment. Hypothetically, it might be supposed that a decreasing oestrogen concentration through vasoconstriction might intensify the prerequisites for the release of tissue activator from the cells and possibly bring about an increased activator concentration in the cells.

Chapter IV

FIBRINOLYTIC ACTIVITY OF SECRETIONS AND TRANSUDATES

Introduction

Few investigations are available on the fibrinolytic activity of body fluids other than blood. However, the knowledge of the fibrinolytic system of the blood and the methods which have been elaborated for its analysis have now also rendered it possible to investigate other body fluids. The analytic methods which were used in the studies considered below are based on the fibrin-plate technique (*Astrup* and *Müllertz* 1952) and on the observation that the plasminogen which is present in the fibrin plates is destroyed by heating to 80° C. for 35 minutes (*Lassen* 1952). By these methods it is possible to distinguish between fibrinolytic activity caused by fibrinolytic enzymes and that due to plasminogen activators. The studies considered below (*Astrup* and *Sterndorff* 1952 b, 1953, *Storm* 1955, *Albrechtsen* and *Thaysen* 1955, *Albrechtsen* and *Trolle* 1955, *Albrechtsen, Storm* and *Claassen* 1958) were all performed by largely the same technique. The ability of the analysed substrates to digest heated bovine fibrin was taken as evidence of the presence of proteolytic enzymes (plasmin). An increase in the fibrinolytic activity against heated bovine fibrin after addition of plasminogen (prepared as described by *Astrup* and *Sterndorff* (1952 b)), which is inactive in itself, was taken as evidence of the presence of plasminogen activators in the substrates. A further increase in the fibrin-splitting action on heated bovine fibrin after addition of both streptokinase and plasminogen to the substrates shows the presence of plasminogen pro-activators (*Müllertz* and *Lassen* 1953). In some experiments, the presence of plasminogen was demonstrated by addition of plasminogen activators

(prepared from human urine as described by *Astrup* and *Sterndorff* (1952 b) or by *Ploug* and *Kjeldgaard* (1956)) to the substrates and measurements of the amounts of plasmin formed on the heated plates. An increase in the fibrinolytic activity of the substrates against heated fibrin after addition of streptokinase, which is inactive in itself, also shows the presence of plasminogen which has been converted into plasmin by the plasminogen activator formed by the streptokinase. Fibrinolytic inhibitors occurring in the analysed substrates were estimated by their ability to inhibit the fibrinolytic action of trypsin on plasminogen-free, heated bovine fibrin.

Fibrinolytic Enzyme Systems of Secretions (Milk, Tears, Saliva, Sweat) and of Urine

Milk.—Milk and casein contain small amounts of a proteolytic enzyme (*Warner* and *Polis* 1945). The proteolytic activity is characterised by its thermolability and by a reaction optimum at about pH 9.2. Addition of streptokinase increases the proteolytic activity in a mixture of milk and rabbit plasma (*Geiger* 1952). These observations show the presence of a fibrinolytic system in milk. A detailed account of this system was given by *Astrup* and *Sterndorff* (1953). While only a trace of activity was found when the effect of human milk on heated fibrin plates was studied, they observed sometimes considerable activity against plasminogen-containing bovine fibrin. The activity increased further on addition of streptokinase. These results were verified by incubation of a mixture of milk, streptokinase and plasminogen for 30 minutes and measurements of the ability of this mixture to digest casein, evidencing the presence of plasmin. It has thus been shown that human milk contains variable amounts of a plasminogen activator and considerable amounts of a plasminogen pro-activator, which can be activated by addition of streptokinase. They found only very small amounts of trypsin inhibitors; this is in agreement with the observation that shaking with chloroform does not produce fibrinolytic activity in milk, whereas the same procedure gives rise to the formation of proteolytic enzymes in blood (*Christensen* 1947), presumably, in part, as a consequence of the destruction of the fibrinolytic inhibitors of the blood.

Tears.—Human tears also contain a fibrinolytic enzyme system

(*Storm* 1955). The lacrimal fluid cannot digest heated bovine fibrin, but may, in certain cases, lyse untreated plasminogen-containing fibrin. This shows the presence of variable, but usually small, amounts of plasminogen activators. Addition of streptokinase caused an increase in the fibrinolytic activity against untreated fibrin; accordingly, the lacrimal fluid also contains a plasminogen pro-activator. In addition, tears contain variable, but usually extremely small, amounts of trypsin inhibitors which can be destroyed by heating to 50° C. for 30 minutes. The plasminogen activator formed by addition of streptokinase is labile, especially at acid and alkaline reactions and after heating to 70 or 100° C., and is thus in that respect similar to that of the blood.

Saliva.—While *Huggins* and *Neal* (1942) failed to reveal proteolytic activity in a mixture of saliva and blood or plasma after incubation at 37° C., *Vergin* (1950) demonstrated the presence of protein-splitting enzymes in the secretion from the parotid gland. Later studies (*Geiger* 1952) showed that saliva contains a thermostable factor which in a mixture of streptokinase and a thermolabile factor from serum or plasma is capable of transforming plasminogen into plasmin. A detailed account of the fibrinolytic enzyme system of saliva was given in studies by *Albrechtsen* and *Thaysen* (1955). Human saliva was collected in small plastic cups placed over the orifice of the parotid duct by a method described by *Curby* (1953). The saliva was collected both before and after stimulation of the gland. While no proteolytic activity against heated bovine fibrin could be demonstrated, variable activity was observed by measurements on the same secretion, both against untreated and heated fibrin, on addition of plasminogen. These results show that a plasminogen activator is present. The activity was most pronounced in unstimulated secretion. Addition of streptokinase to the secretion and measurements on this mixture against plasminogen-containing fibrin revealed an increase in the activity, evidencing that a plasminogen pro-activator was present. The stability of the plasminogen activator formed by streptokinase showed good agreement with that of the plasminogen activator in lacrimal fluid in that it was destroyed at acid and alkaline pH values and at temperatures of 70 and 100° C. It was further shown that parotid saliva does not contain trypsin inhibitors or plasminogen.

Sweat.—Human sweat does not contain fibrinolytic enzymes, plasminogen activators, plasminogen pro-activators or trypsin inhibitors (*Albrechtsen, Storm* and *Claassen* 1958). The sweat samples used in the experiments were collected in a rubber bag placed around one arm of the subject while the other arm was warmed. The complete absence of fibrinolytic components in this secretion may be due to the vigorous stimulation, as it was seen in the experiments with parotid secretion that stimulation leads to a reduction in the activity of the secretion (*Albrechtsen* and *Thaysen* 1955). It is therefore possible that the concentration of active components had become so low that the measuring method used for their recording was not sufficiently sensitive.

Urine.—A number of investigations on the presence of fibrinolytically active components in urine are on record. These have recently been reviewed by *Astrup* (1956 d) and will therefore be only briefly considered here. While previously opinions were rather divergent as to the proteolytic properties of urine, a series of experimental data have now proved that a plasminogen activator is present in urine (*Williams* 1951, *Astrup* and *Sterndorff* 1952 b, *Sobel, Mohler, Jones, Dowdy* and *Guest* 1952). Moreover, variable, although usually small, amounts of a trypsin inhibitor have occasionally been observed in urine. This inhibitor seems to be specific for trypsin and exerts only a slight effect on various proteases and plasminogen activators (*Astrup* and *Sterndorff* 1955), and it is now possible to separate it from the activator. In certain pathological conditions the urinary concentration of the inhibitor is increased. Thus, *Faarvang* (1957) studied the urinary excretion of the trypsin inhibitor in normal subjects, pregnant women and a number of patients with various diseases. The urinary excretion is increased during pregnancy, in patients with febrile diseases and in patients with rheumatoid arthritis under gold or ACTH therapy. The increased excretion is presumably part of a general stress reaction.

While the first investigations (*Astrup* and *Sterndorff* 1952 b) showed that the plasminogen activator of urine was labile, especially at acid reaction, later experiments with purified preparations have demonstrated that the activator is stable at pH levels ranging from 1 to 10 on heating to 50° C. for 30 minutes (*Ploug* and *Kjeldgaard* 1956, 1957). The explanation of this discrepancy must be sought in

the urinary content of pepsinogen, which at acid reaction is converted into pepsin and destroys the plasminogen activator (*Alkjær* and *Astrup* 1957). After removal of the pepsin, the plasminogen activator proved to be stable at acid reaction. In contrast to the plasminogen activator present in secretions and in certain of the below-mentioned transudates, the plasminogen activator of urine thus shows agreement with the tissue activator with regard to thermostability.

The fibrinolytic enzyme system of the secretions differs from that of the blood in that the secretions under normal conditions contain little or no trypsin inhibitors, while these inhibitors are present in the blood in large amounts. It has therefore been discussed whether the fibrinolytic components of the secretions originate from the blood or are secretory products from the gland concerned. Theoretically, both possibilities are open. If the active components are derived from the blood by a diffusion process, it must be assumed that the glandular epithelium is selectively permeable to the active components (plasminogen activators and pro-activators), but is able to retain the trypsin inhibitors. That it is not due to simple filtration appears from the difference in the contents of plasminogen activator observed in secretions from stimulated and unstimulated parotid glands and from the absence of inhibitors. The second possibility, viz. that the active fibrinolytic components should be secretory products from the gland itself, seems less likely. The fall in the activator concentration observed during stimulation might then be thought to be a manifestation of an exhaustion of the gland in the same way as has been seen in other enzymes of the saliva and other secretions (*Babkin* 1950).

Urine differs from milk, tears, saliva and sweat in that it contains a plasminogen activator of the stable type. Hypothetically, this activator might be thought to originate from the tissue-activator-containing kidney. However, the kidney shows little activity. There are thus certain points of similarity between the fibrinolytic systems of the urine and of seminal fluid. Both fluids contain large amounts of stable plasminogen activators and variable amounts of trypsin inhibitors. In addition, it is characteristic of both that addition of streptokinase only in exceptional cases increases the fibrinolytic activity.

The physiological significance of the above-mentioned enzyme

systems of the secretions is unknown. After having revealed the presence of plasminogen activators in the urine, *Astrup* and *Sterndorff* (1952 b) advanced the theory that the fibrinolytic system of the urine should be of importance in the redissolution of fibrin clots, if any, in the urinary tract, in this way securing an unobstructed passage. Similar views have been expressed with regard to the other secretions, which must also pass through relatively narrow ducts. The presence of such fibrin clots in the excretory ducts of the glands, arisen as a result of minor traumata, etc., will result in stagnation of the secretions, as has, for example, been seen in the parotid duct in sialodochitis fibrinosa (*Kussmaul* 1879). The persistence of these fibrin clots must be due either to a decrease in the fibrinolytic power of the secretion or to an increase in the inhibitor concentration.

Fibrinolytic Enzyme Systems of Normal Transudates (Amniotic Fluid, Cerebrospinal Fluid)

Amniotic fluid.—The study of the content of both coagulant and fibrinolytic components in amniotic fluid has been very intensive, especially because great importance has been attached to the part which this fluid plays in the development of the haemorrhagic diathesis which is often observed in relation to amniotic-fluid infusion.

It is an old observation that amniotic fluid is capable of clotting fibrinogen-containing hydrocele fluid (*Bondi* 1903). This coagulant power was later observed by *Russmann* (1914) and *Maeda* (1924). A mixture of amniotic fluid and fresh venous blood has a shorter coagulation time than control samples without amniotic fluid (*Weiner, Reid* and *Roby* 1949). Furthermore, amniotic fluid is capable of clotting recalcified oxalate plasma, but not ordinary oxalate plasma or prothrombin-free oxalate plasma to which calcium and thromboplastin have been added. The amniotic fluid should thus contain thromboplastin, but neither thrombin nor prothrombin. These results have been partially confirmed by *Rendelstein* (1950), *Schneider* (1950), *Rendelstein, Frischauf* and *Deutsch* (1951) and *Szirmai* (1955), but general agreement does not exist as to the thromboplastin concentration.

Bondi's old observation that amniotic fluid after addition of hydrochloric acid can dissolve fibrin, in particular, at body temperature, has not been confirmed (*Maeda* 1924). Nor can fibrin be dissolved by amniotic fluid from normal pregnant women (*Weiner, Reid* and *Roby* 1949) or from women with intra-uterine foetal death (*Hodgkinson, Margulis* and *Luzadre* 1954, *Pritchard* and *Ratnoff* 1955). On the other hand, *Rendelstein, Frischauf* and *Deutsch* (1951) revealed fibrinolytic inhibitors in the amniotic fluid. While amniotic fluid is thus in itself fibrinolytically inactive, addition of streptokinase results in an intense fibrinolytic activity against clotted plasma (*Vecchietti* 1954). This observation forms the basis for the theory that the amniotic fluid contains plasminogen in a concentration corresponding to 2 % of that of the maternal serum. According to our present conception of streptokinase as an activator of the pro-activator of plasminogen, this theory is incorrect. Renewed investigations on this question and on the problem whether or not fibrinolytic inhibitors are present in amniotic fluid are required. *Albrechtsen* and *Trolle* (1955) thus showed that normal amniotic fluid does not contain fibrinolytic enzymes or plasminogen activators (demonstrated by the lack of fibrinolytic effect on both heated and untreated bovine fibrin). On the other hand, addition of streptokinase led to considerable activity against untreated bovine fibrin, but showed only slight effect on heated bovine fibrin, which shows that large amounts of plasminogen pro-activators and only small amounts of plasminogen are present. Moreover, trypsin inhibitors were found to be present in a high concentration. Both the plasminogen activator formed by the streptokinase and the trypsin inhibitors were labile, especially at acid reaction. The fibrinolytic enzyme system of amniotic fluid thus corresponds to that of normal inactive blood.

As emphasised by *Rendelstein* (1950), bleedings from vaginal and vulval tears are arrested strikingly rapidly after delivery, even in the absence of surgical haemostasis. The thromboplastic content of the amniotic fluid is claimed to play a part in this haemostasis and likewise in postpartum haemostasis (*Weiner, Reid* and *Roby* 1949). In addition, *Szirmai* (1955) observed increasing concentrations of coagulant components in amniotic fluid from thrombophilic

patients; this might be the reason why postpartum haemorrhages largely do not occur more frequently in such patients than in normal parturient women.

The influence of the amniotic fluid on the circulating blood during the course of so-called amniotic-fluid infusion (amniotic embolism) has also been the subject of discussion. This obstetrical complication has been described repeatedly (*Steiner* and *Lushbaugh* 1941, *Ahlström* and *Widlund* 1952, *Albrechtsen, Storm* and *Trolle* 1955 a, b, and others). The condition is characterised by a shock of sudden onset developing during labour or during the first few hours after delivery, sometimes ushered in by a short initial stage with anxiety, unrest and vomiting. The patient becomes dyspnoeic and cyanotic and shows signs of pulmonary oedema. The pulse becomes weak and later impalpable, and the blood pressure falls. The prognosis is grave, death often occurring during the acute shock. In the patients who survive this initial phase, haemorrhagic diathesis, with persistent profuse uterine bleedings, and occasionally also a generalised tendency to bleeding develop (bleedings from the mucosae, ecchymoses). In the absence of the institution of adequate therapy, this haemorrhagic diathesis often leads to death.

The aetiology is only partially known. Components of amniotic fluid have been observed in the pulmonary arterioles and capillaries and in the sinusoids of the uterine wall, indicating that amniotic fluid has escaped from the uterus into the circulating blood (*Steiner* and *Lushbaugh* 1941, and others). It has been claimed that a causal relationship exists between this amniotic-fluid infusion and the primary shock. However, the blockage observed in the pulmonary circulation has in most cases been so slight that it cannot account for the shock, for which reason others have explained its occurrence on the basis of vascular spasms or as an allergic reaction excited by the infused proteins.

On the other hand, *Weiner* and *Reid* (1950) emphasised that the exciting factor might be the thromboplastin content of the amniotic fluid, which should give rise to intravascular transformation of the blood fibrinogen into fibrin, so that smaller or larger fibrin emboli in the pulmonary circulation should be the cause of the shock. However, it has not been possible to confirm this theory by

post-mortem examinations, at which such pulmonary emboli have never been demonstrated, but it cannot be ruled out that such fibrin emboli may have been redissolved post mortem (see below).

In all the cases on record, the subsequent haemorrhagic diathesis has been caused by an afibrinogenaemia, the aetiology of which is likewise unknown. *Schneider* (1953) voiced the opinion that the afibrinogenaemia should be due to hyperheparinaemia excited by the primary shock by a liberation of heparin from the mast cells, while *Weiner* and *Reid* (1950) assumed that the trigger mechanism was to be sought in infusion of thromboplastin resulting in an intravascular transformation of the blood fibrinogen into fibrin. Others have expressed the view that the afibrinogenaemia was due to the presence of fibrinolytic activity in the blood. Such an increased fibrinolysis has repeatedly been observed (*Ratnoff* and *Vosburgh* 1952, *Reid, Weiner* and *Roby* 1953 a, b), and it has been shown that this increased fibrinolysis is due to the presence of a plasminogen activator (*Albrechtsen, Storm* and *Trolle* 1955 a, b). The mechanism of the activation of fibrinolysis in these patients is unknown. It is possible that it in some unknown manner is excited by the primary shock, since increased fibrinolytic activity in the blood has previously been observed in shock (see *Macfarlane* and *Biggs* 1948). In this connexion it is of interest to point out that in the patient described by *Albrechtsen, Storm* and *Trolle* the blood did not contain plasminogen pro-activators, since the fibrinolytic activity of the blood could not be increased by addition of streptokinase. It was therefore suggested that a lysokinase of unknown origin was liberated during the shock and activated fibrinolysis. *Vecchietti* (1953) assumed that the amniotic fluid from these patients should be fibrinolytically active, and that this activity, owing to the infusion, should come into play in the blood, but this assumption was opposed by *Pritchard* and *Ratnoff* (1955) and *Hodgkinson, Margulis* and *Luzadre* (1954). Hypothetically, a local activation of the pro-activator of the amniotic fluid and a subsequent infusion into the circulating blood will be able to explain this fibrinolysis.

The treatment of patients with amniotic-fluid infusion has two phases. In the light of our present knowledge it will be correct to administer oxygen in the treatment of the primary shock and, if necessary, to assist the respiration with oxygen under positive pressure

in order to prevent anoxaemia. In order to counteract spasms in the pulmonary circulation procaine and eupaverin injections should be given a trial. If the patient survives the acute shock, the next problem consists in preventing the occurrence of further shocks and in treating the haemorrhagic diathesis. The lines on which this latter phase of the therapy should be directed are the same as have previously been outlined in the treatment of hypo- and afibrinogenaemia in other obstetrical complications (premature separation of the placenta, intra-uterine foetal death, etc.).

Cerebrospinal fluid.—While *Huggins* and *Neal* (1942) did not observe any signs of fibrinolytic activity of the cerebrospinal fluid when this was tested on human blood or plasma, *Halse* (1948) demonstrated a weak fibrin-splitting action on human fibrin. *Bierstedt* (1955 a, b, c) later observed similar fibrinolytic properties of cerebrospinal fluid withdrawn from human cadavers. It is difficult to explain the discrepancy between the studies of *Huggins* and *Neal* and *Halse,* since they used the same substrates. The results of *Huggins* and *Neal* were confirmed in renewed investigations by *Albrechtsen, Storm* and *Claassen* (1958), who did not find any fibrinolytic activity of the cerebrospinal fluid either against heated or untreated bovine fibrin; this shows that both fibrinolytic enzymes and plasminogen activators are absent. Addition of streptokinase resulted in fibrinolytic activity against plasminogen-containing fibrin, which shows that plasminogen pro-activators are present. The plasminogen activator formed in this way was labile, especially at acid reaction, and is thus similar to the labile plasminogen activator of the blood. Trypsin inhibitors were not revealed in the cerebrospinal fluid; this observation is in agreement with *Rosenmann*'s old finding (1922), viz. that cerebrospinal fluid cannot inhibit spontaneous fibrinolysis in precipitated and washed horse fibrin. Addition of plasminogen activator from human urine did not result in the formation of plasmin, i. e. plasminogen was absent.

The fibrinolytic enzyme system of the cerebrospinal fluid is thus similar to that of the secretions of the human organism. As the cerebrospinal fluid, just like the secretions, is to pass through narrow ducts, its fibrinolytic system may, as has also been claimed with regard to those of the secretions, be of significance for the dissolution of fibrin clots, if any, and thus be instrumental in securing an

unimpeded passage. Streptokinase injections have been used in the treatment of fibrinous exudates in tuberculous meningitis. By the dissolution of intrathecal fibrin clots and adhesions, this treatment counteracts an increase in the intracranial pressure and intensifies the effect of antibiotics (*Cathie* and *Macfarlane* 1950). The effect observed in this therapy is thus presumably due to an activation of the plasminogen pro-activator of the cerebrospinal fluid.

Fibrinolytic Enzyme Systems of Pathological Transudates (Ascitic, Synovial, Hydrocele and Blister Fluids and Lymph)

Ascitic fluid.—Halse (1948) observed that ascitic fluid can lyse human fibrin, and this observation was confirmed by *Bierstedt* (1955 a, b, c). It has later been shown that this fibrinolytic activity is due to a plasminogen activator (*Albrechtsen, Storm* and *Claassen* 1958), since activity was observed only when the ascitic fluid reacted with untreated bovine fibrin containing plasminogen, while it was inactive against heated fibrin. In addition, ascitic fluid contains a plasminogen pro-activator, which can be converted into plasminogen activator on addition of streptokinase, and small amounts of trypsin inhibitors and plasminogen. The latter observation confirms the finding reported by *Rosenmann* (1922), viz. that ascitic fluid contains substances inhibiting spontaneous fibrinolysis in precipitated and washed horse fibrin. The plasminogen activator normally present is labile, especially at acid reaction and at temperatures of 70 and 100° C., and thus corresponds to the plasminogen activator of the blood. The plasminogen activator formed on addition of streptokinase shows a similar lability, for which reason it was concluded that the former activator is presumably formed by an activation of the pro-activator.

Synovial fluid.—It has previously been shown that synovial fluid from traumatic hydrarthrosis (*Halse* 1948) and from cadavers (*Bierstedt* 1955 a, b, c) can lyse human fibrin, although to a smaller extent than ascitic fluid. Our own investigations (*Albrechtsen, Storm* and *Claassen* 1958) did not confirm these observations; synovial fluid aspirated from patients suffering from acute, uninflamed hydrarthrosis was unable to digest untreated or heated bovine fibrin. Addition of streptokinase resulted in a considerable activity against

untreated fibrin, indicating the presence of a plasminogen pro-activator. Moreover, plasminogen and trypsin inhibitors were found to be present in synovial fluid. The stability of the plasminogen activator formed on addition of streptokinase was of the same nature as that of the labile activator in ascitic fluid and blood, since it was destroyed at acid reaction and temperatures of 70 and 100° C.

Fluids from hydroceles and blisters, lymph, etc.—Pleural fluid and hydrocele fluid can digest human fibrin *(Halse* 1948, *Bierstedt* 1955 a, b, c). In addition, pleural fluid contains fibrinolytic inhibitors, especially if it is of tuberculous origin *(Rosenmann* 1922). Our own investigations (*Albrechtsen, Storm* and *Claassen* 1958) showed that the fibrinolytic systems of hydrocele fluid, blister fluid and lymph closely correspond to those observed in the other pathological transudates. Thus, they contained plasminogen pro-activators and trypsin inhibitors, but not plasmin or plasminogen activators. *Macfarlane* (1943) arrived at a similar result in his study of blister fluid.

The fibrinolytic systems of both normal and pathological transudates closely correspond to that of the blood. However, cerebrospinal fluid differs from the other transudates in that trypsin inhibitors are absent. The fibrinolytic components observed in the transudates presumably originate from the blood.

It is a well-known fact that blood which somehow enters the serous cavities of the body after coagulation again becomes liquid. This process is of great importance in the treatment of such cases, since a simple puncture of the serous cavity concerned is sufficient to restore normal conditions. The cause of the redissolution of the fibrin clots formed must presumably be sought in the fibrinolytic potential which is mentioned here, but obviously also to a great extent in the fibrinolytic components of the blood. Fibrinolysis is thus of significance in counteracting fibrin deposits and subsequent formation of connective tissue. This physiological process may be further intensified by injection of fibrinolytic activators. It has thus been claimed that streptokinase is capable of counteracting or dissolving fibrin deposits and adhesions formed in the peritoneal cavity (*Wright, Smith, Rothman, Quash* and *Metzger* 1950), but general agreement as to this therapy has not been attained. According to the report published by *Gustavsson, Blombäck, Blombäck* and *Wallen* (1955),

Table 7.

Content of plasminogen pro-activators, plasminogen activators and trypsin inhibitors in secretions and in normal and pathological transudates.

	Pro-activator	Activator	Inhibitor
Normal inactive blood	+	0	+
Spontaneously active blood	+	+	+
Milk	+	(+)	0
Saliva	+	(+)	0
Tears	+	(+)	0
Sweat	(0)	(0)	(0)
Seminal fluid	(+)	+	+
Cerebrospinal fluid	+	0	0
Amniotic fluid	+	0	+
Ascitic fluid	+	(+)	+
Lymph	+	0	+
Synovial fluid	+	0	+
Blister fluid	+	0	+
Hydrocele fluid	+	0	+

only the enzyme plasmin should be active, whereas streptokinase should be without any effect.

Table 7 gives a survey of the observed content of plasminogen pro-activators, plasminogen activators and trypsin inhibitors in various body fluids as compared with inactive blood and spontaneously active blood.

Chapter V

CONCLUSIVE REMARKS

The components of the fibrinolytic enzyme system and their interrelationship are now known in broad outline. The transformation of the plasminogen pro-activators into plasminogen activators under the influence of lysokinases and the transformation of plasminogen into plasmin under the influence of plasminogen activators have been analysed *in vitro*. Moreover, investigations performed during recent years have revealed the significance of fibrinolysis in a number of physiological and pathological processes. Weighty evidence suggests that we are here facing a process of vital importance. However, it has not as yet been unquestionably shown how and under what conditions fibrinolysis is activated *in vivo*. The conversion of the plasminogen of the blood into the active enzyme plasmin presupposes the presence of plasminogen activators. In vitro, these activators may be formed from pro-activators which are normally present, but it has not as yet been possible with certainty to demonstrate an activator for the latter (i. e. lysokinase) in the normal organism. It has been of great importance in the analyses of the composition of the system to ascertain that streptokinase acts as a lysokinase, but this observation has not shed further light on the mechanism in vivo.

At the present time, we know only relatively few fibrinolytic activators in the organism. Among these, the tissue activator of plasminogen seems to play a predominant part. In the present monograph, its distribution in the organism has been considered, and its importance in the activation of the fibrinolytic system in vivo has been discussed. The plasminogen present in the blood (and in fibrin deposits, if any) can only be activated by means of the tissue activator if contact is established between the intravascular and

intracellular spaces. As several investigators have shown that the tissue activator is firmly bound to the tissue proteins and can be extracted from these only by means of certain solvents, such a contact seems to be impossible, and, accordingly, the tissue activator has been regarded as a component with only a local effect in the organism. One of the purposes of the present investigations has been to analyse this problem in detail. As the starting point of these investigations, it was chosen to analyse the fibrinolytic conditions in an anatomically well-defined region. As many previous studies had shown that the uterus is the site of a number of fibrinolytic processes whose nature was previously unknown, this organ seemed to be a suitable object of the study. It appears that a plasminogen activator is present in the endometrium, and that this activator during menstruation and the attendant cell necrosis is liberated from the cells and exerts its action on the menstrual blood. The fact that the tissue activator under certain conditions can be liberated from the tissues in a local region renders it possible that a similar process may come into play in other regions under corresponding conditions. Later investigations have also shown that the plasminogen activator present in the prostate may also be liberated from the gland. Finally, it has now been demonstrated that the tissue activator of plasminogen may to some extent be extracted by means of physiological saline. As saline extraction approaches a physiological process to a greater extent than similar extractions with special solvents, such as potassium thiocyanate, there are reasons to believe that the activity of the saline extracts reflects the immediate availability of the plasminogen activator for the organism.

However, these considerations do not exclude the possibility that the fibrinolytic components of the blood may also be activated by the aforementioned mechanism in which the pro-activator of the blood is transformed into the activator under the influence of lysokinases. The conclusive proof of the correctness of this theory can be provided only by the demonstration of such lysokinases in the organism. The presence of such lysokinases will also be necessary if importance is to be attached to the fibrinolytic systems of secretions and transudates in the dissolution of fibrin deposits in the excretory ducts of the glands and in the serous cavities of the body, since these body fluids contain pro-activators, but usually not activators.

The interrelationship which has now been demonstrated between the fibrinolytic activity in the uterus and the female sex hormones opens a possibility of continued studies, which may throw light on previously unexplained bleedings in both pregnant women and women with irregular menstruation. So far, we are still unable to give effective treatment in certain bleeding anomalies of either a general or local nature encountered during pregnancy and delivery. Bleedings play a predominant role within the field of obstetrics. The fibrinogen therapy marks an immense step forward in the treatment of these bleeding anomalies, but a greater understanding and an effective therapy of the bleeding anomalies which may be referable to fibrinolysis are required to attain a further reduction in the mortality among parturient women.

SUMMARY

The purpose of the present monograph is to give an account of the distribution of fibrinolytic components in the organism, with special reference to the tissue activator of plasminogen.

Fibrinolytic activity of tissues.—Various tissues from man and animals can digest fibrin. It has previously been shown that this digestion is referable to an activator present in the tissues; this activator is capable of transforming the fibrinolytic pro-enzyme, plasminogen, which is present in fibrin, into the active fibrinolytic enzyme, plasmin.

Several tissues also contain components with an inhibitory effect on the fibrinolytic process. The nature of most of these components is unknown. However, it is known that a low-molecular plasmin inhibitor (pulmin) is present in tissues from the bovine lung and uterus, and it has been shown that no other tissues contain pulmin.

Great difficulties have been encountered in elaborating methods for qualitative and quantitative determinations of the concentration of the tissue activator in fresh tissues, particularly because the tissue activator is firmly bound to the tissue proteins. A review of previously used analytic methods shows that they all had some qualitative or quantitative shortcomings. The elaboration of an analytic method for the quantitative determination of the content of plasminogen activators in the tissues was therefore a prerequisite for the present study. Based on the observation that potassium thiocyanate is a specific solvent for the extraction of the tissue activator it was possible to elaborate such a method, by which the concentration of the tissue activator could be expressed in terms of arbitrary units. The tissue concentration of pulmin may also be determined by this method.

The plasminogen activator of the tissues is relatively stable and

differs in that respect from the plasminogen activator present in the blood under certain conditions. Thus, it is not destroyed by heating to 70 or 100° C. at acid reaction, and it is stable at 37 and 50° C. within a very wide pH range. It is also stable to the action of a large number of chemicals, but is destroyed by formalin. It is very firmly bound to the tissue proteins and can be extracted only by repeated treatments with potassium thiocyanate. The activator is presumably chiefly localised in the microsomes.

A number of studies have shown that the tissue activator presumably reacts stoicheiometrically with plasminogen in an equilibrium process.

Quantitative determinations of the content of tissue activator have revealed considerable variations from organ to organ in the same animal, from animal to animal of the same species, and from one animal species to another. These variations are at present unexplained.

Wide variations in the concentration of the tissue activator have also been revealed in various human tissues, but it is possible to classify the organs into groups with high, moderate and low concentrations. The first group comprises such organs as the uterus, adrenals, lymph nodes, thyroid gland, lungs, prostate and ovaries, whereas liver tissue is practically inactive.

The significance of the tissue activator is not definitely known. It has often been emphasised that it should be of the greatest importance in the redissolution of fibrin deposits and thus be able to counteract the formation of connective tissue. It has further been claimed that a relationship exists between the tissue activator and the occurrence of local bleedings in the tissues; by causing a redissolution of the fibrin deposits formed for the purpose of haemostasis it should be a contributory factor in the persistence of the bleeding. It is unknown whether or not the tissue activator is of importance in the fibrinolytic activity of the blood in vivo or post mortem. Some experimental data are in favour of the assumption that the tissue activator under certain conditions (tissue destruction, cell necrosis) may be liberated from the cells and enter the circulating blood.

Fibrinolytic activity of the uterus.—Both human and animal myometrial tissues contain a plasminogen activator. Thus, the myo-

metrium is the tissue of the human organism which contains the activator in the highest concentration.

Plasminogen activators are also present in the human endometrium. The concentration varies with the various stages of the menstrual cycle; it is only moderate in the proliferative and intermediate stages, but considerable in the secretory stage. The concentration decreases with age; the senile endometrium does not contain tissue activator. Considerable amounts of tissue activator are present in certain pathological endometria (endometrial hyperplasia, etc.). It is reasonable to assume that a relationship exists between the tissue activator of the endometrium and the female sex hormones. Such a relationship has also been observed in animal experiments, in which it has been shown that a decrease in the blood concentration of oestrogens results in an increase in the uterine concentration of the tissue activator.

Based on these observations the theory has been advanced that the tissue activator is of significance in the occurrence of uterine bleedings. It has thus been thought that the decreasing oestrogen concentration in the circulating blood in the premenstrual phase through a constriction of the spiral artery should give rise to incipient cell necrosis in the endometrium and thus provide optimum conditions for a liberation of the tissue activator. This liberation should be a contributory factor in the occurrence of bleeding from the tissue.

The tissue activator is absent in normal decidual and placental tissues, but present in pathological decidual tissue from spontaneous abortions. It is possible that the tissue activator in this pathological tissue may be a contributory factor in the occurrence of bleeding from the tissue and thus be of importance in the development of spontaneous abortion.

Normal menstrual blood does not clot spontaneously or after addition of thrombin. This is due to the absence of fibrinogen, because the menstrual blood has previously been clotted in the uterine cavity. This coagulation process is presumably due to a liberation of thromboplastin from the endometrium. Subsequent redissolution of the fibrin clot formed in this way is caused by a fibrinolytic process excited by the liberation of the tissue activator from the endometrium. This has been shown by the finding of a stable

plasminogen activator of the tissue-activator type in menstrual blood and by the presence of plasmin and the absence of plasminogen. Thus, the incoagulability of menstrual blood is due to the combined effects of tissue thromboplastin and tissue activator, which have both been liberated from the injured endometrial tissue.

As distinct from the local afibrinogenaemia of menstrual blood, hypo- or afibrinogenaemia has often been observed in the circulating blood, especially as an obstetrical complication. It is not known with certainty whether this condition may also be due to a liberation of tissue components (thromboplastin, tissue activator) from the uterus.

Fibrinolytic activity of the prostate and seminal fluid.—Prostatic tissue is fibrinolytically active. It has been shown that this activity is due to the presence of two plasminogen activators in the tissue, of which one corresponds to the stable plasminogen activator of the tissues and the other to the labile plasminogen activator of the blood.

Seminal fluid likewise contains plasminogen activators. It has been shown that the plasminogen activator of seminal fluid corresponds to the plasminogen activator of the tissues and is stable, for which reason it presumably originates from the prostate. However, the experimental results available do not exclude that the seminal fluid also contains plasminogen activators of the labile type.

Whereas the plasminogen activators of the prostate are thus presumably of importance for the fibrinolytic activity of seminal fluid, it is not yet known with certainty whether they may also give rise to the fibrinolytic activity in the circulating blood which has been observed in cases of cancer of the prostate and after prostatectomy.

Fibrinolytic activity of secretions and transudates.—Previous investigations on the fibrinolytic enzyme system of the blood have now rendered it possible to subject the fibrinolytic systems of other body fluids to a closer analysis.

Some secretions (milk, tears and saliva) contain fibrinolytic enzyme systems which differ from that of the blood. They are characterised by the presence of variable amounts of plasminogen activators and large amounts of plasminogen pro-activators which can be

transformed into the activator itself by addition of streptokinase. As distinct from blood, the secretions do not contain trypsin inhibitors. On the basis of these results it is discussed whether the fibrinolytic components originate from the blood or are secretory products from the gland concerned. The former assumption appears to be more likely. Accordingly, the glandular epithelium must be assumed to be selectively permeable to the active components, but capable of retaining the inhibitors.

A number of normal and pathological transudates also contain fibrinolytic enzyme systems largely corresponding to that of the blood. Thus, they contain large amounts of trypsin inhibitors and plasminogen pro-activators. However, cerebrospinal fluid differs from the other transudates in that it does not contain inhibitors and is thus reminiscent of the secretions. In the present study, special attention was focused on the fibrinolytic enzyme system of amniotic fluid because it may be of significance in the haemorrhagic diatheses which have occasionally been observed in relation to amniotic-fluid infusions.

SAMMENDRAG

Hensigten med det foreliggende arbejde har været at redegøre for fordelingen af fibrinolytiske komponenter i organismen, idet der specielt er lagt vægt på den i vævene forekommende plasminogen aktivator.

Fibrinolytisk aktivitet i væv: En række væv fra såvel mennesker som dyr kan opløse fibrin. Det er tidligere vist, at dette skyldes en i vævene tilstedeværende aktivator, som kan omdanne det i fibrinet tilstedeværende fibrinolytiske proenzym, plasminogen, til det aktive fibrinolytiske enzym plasmin.

En række væv indeholder ligeledes komponenter med en hæmmende effekt overfor den fibrinolytiske proces. Naturen af disse er for størstedelen ukendt. Man har imidlertid kendskab til tilstedeværelsen af en lavmolekylær plasmininhibitor (pulmin) i okselungevæv samt i den bovine uterus, og det er vist, at andre væv ikke indeholder pulmin. Pulminets betydning er ukendt.

Det har været forbundet med betydelige vanskeligheder at foretage kvalitative og kvantitative bestemmelser af vævsaktivatorkoncentrationen i friske væv. Dette skyldes ikke mindst vævsaktivatorens stærke binding til vævsproteinerne. En gennemgang af tidligere analytiske metoder har vist, at de alle frembyder enten kvalitative eller kvantitative mangler. Det har derfor været en forudsætning for det foreliggende arbejde at udarbejde en analysemetode til kvantitativ bestemmelse af vævenes indhold af plasminogen aktivatorer. Baseret på fundet af kaliumrhodanid som et specifikt ekstraktionsmiddel for vævsaktivatoren er det nu lykkedes at udarbejde en sådan metode, ved hjælp af hvilken vævsaktivatorkoncentrationen kan udtrykkes i vævsaktivatorenheder. Denne metode tillader ligeledes bestemmelse af vævenes indhold af pulmin.

Vævenes plasminogen aktivator er relativt stabil og adskiller sig herved fra en i blodet under visse forhold tilstedeværende plasminogen aktivator. Den ødelægges således ikke ved opvarmning til 70 og 100 grader ved sur reaktion og er stabil ved 37 og 50 grader over et meget bredt pH spektrum. Den er stabil overfor påvirkning af en lang række kemiske stoffer, men ødelægges dog af formalin. Den er bundet meget kraftigt til vævsproteinerne og kan kun ekstraheres kvantitativt ved gentagne behandlinger med kaliumrhodanid. Aktivatoren er formentlig hovedsagelig lokaliseret til microsomerne.

En række undersøgelser har vist, at vævsaktivatoren formentlig reagerer støkiometrisk med plasminogen i en ligevægtsproces.

Kvantitative bestemmelser af vævsaktivatorindholdet i dyriske organer har vist betydelige variationer fra organ til organ hos samme dyr, fra dyr til dyr indenfor samme art og fra dyreart til dyreart. Disse variationer kan ikke forklares.

Ligeledes er der fundet betydelige variationer i vævsaktivatorkoncentrationen i humane væv. Det har dog været muligt at skelne mellem organer med et stort, et moderat og et ringe indhold. Den første gruppe omfatter organer som uterus, binyren, lymfekirtlerne, glandula thyreoidea, lungen, prostata og ovariet, medens levervæv er praktisk talt inaktivt.

Vævsaktivatorens betydning er ikke med sikkerhed kendt. Det er hyppigt fremhævet, at den skulle være af den største betydning for genopløsningen af lokale fibrindannelser i vævene og således i stand til at modvirke bindevævsdannelsen. Man har yderligere villet se en forbindelse mellem vævsaktivatoren og forekomsten af lokale blødninger i vævene, idet den ved at forårsage en genopløsning af i hæmostatisk øjemed dannede fibrinlag kunne have betydning for en blødnings fortsatte beståen. Hvorvidt vævsaktivatoren har betydning for opståen af fibrinolytisk aktivitet i blodet såvel in vivo som post mortem vides ikke. Visse eksperimentelle data taler til gunst for, at vævsaktivatoren under visse bestemte betingelser (vævsødelæggelse, cellenekrose) kan frigøres fra cellerne og gå over i det circulerende blod.

Fibrinolytisk aktivitet i uterus: Myometrievæv fra såvel mennesker som dyr indeholder en plasminogen aktivator. Det humane

myometrium er således det mest vævsaktivatorholdige væv i den humane organisme.

Der findes ligeledes plasminogen aktivatorer i det humane endometrium. Koncentrationen af disse varierer med menstruationscyclus og er kun moderat i proliferationsfasen og intermediærfasen, men betydelig i sekretionsfasen. Koncentrationen aftager med alderen, og der findes ingen vævsaktivator i det senile endometrium. Visse pathologiske endometrier (endometriel hyperplasi m. m.) indeholder betydelige mængder vævsaktivator. Det har været naturligt at antage, at der er en forbindelse mellem vævsaktivatoren i endometriet og de kvindelige kønshormoner. En sådan er da også observeret i dyreforsøg, idet det er vist, at en aftagen af østrogenkoncentrationen i blodet medfører en stigning i vævsaktivatorkoncentrationen i uterus.

Disse iagttagelser ligger til grund for den teori, at vævsaktivatoren skulle være af betydning for optræden af uterine blødninger. Man har således ment, at den faldende østrogenkoncentration i det circulerende blod før menstruationen via en kontraktion af a. spiralis skulle forårsage en begyndende nekrose af cellerne i endometriet og dermed etablere de bedste betingelser for en frigørelse af vævsaktivatoren. Denne vævsaktivatorfrigørelse skulle være en medvirkende faktor ved optræden af blødning i vævet.

Der findes ikke vævsaktivator i normalt decidua- og placentarvæv, men derimod i pathologisk deciduavæv fra spontane aborter. Det er muligt, at vævsaktivatoren i dette pathologiske væv kan være en medvirkende faktor ved optræden af blødning i vævet og således have betydning for abortens opståen.

Normalt menstruationsblod kan ikke koagulere hverken spontant eller ved tilsætning af trombin. Dette skyldes en mangel på fibrinogen, idet menstruationsblodet tidligere har været koaguleret i uterincaviteten. Denne koagulationsproces skyldes formentlig en frigørelse af tromboplastin fra endometriet. Til grund for den senere indtrædende genopløsning af det således dannede fibrinkoagel ligger en fibrinolytisk proces, fremkaldt ved frigørelse af vævsaktivatoren fra endometriet. Dette er vist ved fundet af en stabil plasminogen aktivator af vævsaktivatortypen i menstruationsblodet samt ved fundet af plasmin og mangelen på plasminogen. Menstruationsblodets manglende koagulationsevne skyldes således en kombi-

neret effekt af vævstromboplastin og vævsaktivator, begge frigjort fra det læderede endometrievæv.

I modsætning til den lokalt optrædende afibrinogenæmi i menstruationsblodet er der ofte observeret hypo- eller afibrinogenæmi i det circulerende blod, navnlig som obstetrisk komplication. Hvorvidt denne ligeledes skyldes en frigørelse af vævskomponenter fra uterus (tromboplastin, vævsaktivator) vides ikke med sikkerhed.

Fibrinolytisk aktivitet i prostata og sperma: Prostatavæv er fibrinolytisk aktivt. Det er vist, at denne aktivitet skyldes tilstedeværelsen af to plasminogen aktivatorer i vævet, hvoraf den ene svarer til vævenes stabile plasminogen aktivator og den anden til blodets labile plasminogen aktivator.

Sperma indeholder ligeledes plasminogen aktivatorer. Det er vist, at plasminogen aktivatoren i sperma svarer til vævenes plasminogen aktivator og er stabil, hvorfor den formentligt må stamme fra prostata. Dog udelukker de foreliggende forsøg ikke, at der samtidig findes plasminogen aktivatorer af den labile type i sperma.

Medens plasminogen aktivatorerne i prostata således formentlig er af betydning for den fibrinolytiske aktivitet i sperma, vides det endnu ikke med sikkerhed, hvorvidt de ligeledes kan forårsage fibrinolytisk aktivitet i det circulerende blod, således som det er observeret ved tilfælde af cancer prostatae og efter prostatectomier.

Den fibrinolytiske aktivitet i sekreter og transsudater: Tidligere undersøgelser af blodets fibrinolytiske enzymsystem har nu muliggjort en nærmere analyse af de fibrinolytiske systemer i andre legemsvædsker.

En række sekreter (mælk, tårer og spyt) indeholder fibrinolytiske enzymsystemer, som adskiller sig fra blodets enzymsystem. De er karakteriseret ved tilstedeværelsen af varierende mængder plasminogen aktivatorer og store mængder plasminogen proaktivatorer, som kan omdannes til selve aktivatoren ved tilsætning af streptokinase. Der findes, i modsætning til hvad der er tilfældet i blodet, ingen trypsininhibitorer i sekreterne. På grundlag af disse resultater har man diskuteret, hvorvidt de fibrinolytiske komponenter stammer fra blodet eller er sekretionsprodukter fra kirtlen. Den første teori forekommer mest sandsynlig, hvorfor kirtelepithelet må anta-

ges at være selektivt permeabelt for de aktive komponenter, men i stand til at tilbageholde inhibitorerne.

En række transsudater og pathologiske transsudater indeholder ligeledes fibrinolytiske enzymsystemer, som stort set svarer til enzymsystemet i blodet. Der findes således store mængder trypsininhibitorer og plasminogen proaktivatorer. Imidlertid adskiller cerebrospinalvædsken sig fra de øvrige transsudater ved ikke at indeholde inhibitorer og minder således om sekreterne. Interessen i det foreliggende arbejde har navnlig koncentreret sig om amnionvædskens fibrinolytiske enzymsystem på grund af dettes eventuelle betydning for de hæmorrhagiske diatheser, der i flere tilfælde er observeret i tilslutning til amnioninfusioner.

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